

The status of the predatory mite *Neoseiulus californicus* (McGregor)
(Acari: Phytoseiidae) in the UK, and its potential as a biocontrol agent
of *Panonychus ulmi* (Koch) (Acari: Tetranychidae)

by

Rebecca Louise Jolly

A thesis submitted to The University of Birmingham
for the degree of DOCTOR OF PHILOSOPHY

Entomology & Plant
Pathology Department
Horticulture Research International
East Malling, Kent

School of Biosciences
The University of Birmingham
February 2001

UNIVERSITY OF
BIRMINGHAM

University of Birmingham Research Archive

e-theses repository

This unpublished thesis/dissertation is copyright of the author and/or third parties. The intellectual property rights of the author or third parties in respect of this work are as defined by The Copyright Designs and Patents Act 1988 or as modified by any successor legislation.

Any use made of information contained in this thesis/dissertation must be in accordance with that legislation and must be properly acknowledged. Further distribution or reproduction in any format is prohibited without the permission of the copyright holder.

ABSTRACT

The non-native predatory phytoseiid mite *Neoseiulus californicus* has been found in recent years in UK apple orchards. The aims of this study were to determine whether this mite could establish in the UK and its potential as a biocontrol agent for *Panonychus ulmi*.

By reviewing the literature and examining specimens of *N. californicus*, it was concluded that taxonomic synonymies with *Amblyseius californicus*, *Amblyseius chilensis* and *Typhlodromus mungeri* could be supported, but those with *Typhlodromus marinus* and *Neoseiulus fallacis* could not.

Neoseiulus californicus was found in strawberry, hop, blackcurrant and apple plantations in the main fruit growing regions of the UK. Field and laboratory studies showed that *N. californicus* possesses the ability to diapause, is a chill tolerant species and can survive winter field conditions in the UK.

Neoseiulus californicus was found to readily consume both *Panonychus ulmi* and *Tetranychus urticae* and consumed greater numbers of prey than the native phytoseiid *Typhlodromus pyri*. Deutonymphs consumed an average of 1.8 and 1.6 immature *P. ulmi* stages per day respectively and an average of 2.6 and 1.4 *T. urticae* respectively. The total mean development time for *N. californicus* was 7.47 days and for *T. pyri* was 12.45, feeding on *P. ulmi*.

Neoseiulus californicus from USA, Spain and UK displayed differences in measurements of a selection of morphological characteristics, diapause ability (16, 0 and 96% diapause respectively), development times (shortest for USA and longest for UK), fecundity (0.82-0.97 eggs per day) and esterase banding patterns, indicating the existence of different detectable strains.

In conclusion, *N. californicus* was found to be a component of fruit plantation fauna in the UK, has the potential to survive winter field conditions and readily consumes *P. ulmi* and *T. urticae*.



ACKNOWLEDGEMENTS

I would like to thank especially Dr Mike Solomon and Dr Jean Fitzgerald, my supervisors at Horticulture Research International, for their encouragement and advice with the work for this thesis.

Thanks are also due to Prof. Jeff Bale, my supervisor at Birmingham University. To Pete Squires and Wilma Reerink, Koppert Ltd, for providing *N. californicus* in swift response to my requests for them. Thank you to Dick Simpson for acting as courier for some American *N. californicus*. Dr Roger Worland, BAS, for allowing me to spend several days using the DSC. Shelagh Reardon, Wye College, for allowing me time on the SEM. Dr. Colin Campbell for statistics advice and John Fenlon for some statistical analyses. Penny Greeves for graphics assistance.

In addition, I would like to thank James Everett for writing an Excel Macro that facilitated the extraction of development rates from my data and Alex Dowdeswell for helping check my references and proof reading. Thanks too to Alex, Lucy Collinson and my family for their support.

This work was funded by the East Malling Trust for Horticultural Research (Blackman Studentship).

TABLE OF CONTENTS

CHAPTER 1: INTRODUCTION	1
1.1 Introduction	1
1.1.1 <i>Biological characteristics of N. californicus</i>	1
1.1.2 <i>Effect of prey type on the biology of N. californicus</i>	4
1.1.3 <i>Effect of pesticides on N. californicus</i>	6
1.1.3.1 <i>Acaricides</i>	6
1.1.3.2 <i>Insecticides</i>	7
1.1.3.3 <i>Fungicides</i>	9
1.1.4 <i>The potential role of N. californicus as a biocontrol agent of P. ulmi</i>	14
 CHAPTER 2: TAXONOMY	 16
2.1 Introduction	16
2.2 Review of the taxonomic history of <i>N. californicus</i>	18
2.2.1 <i>Typhlodromus mungeri</i>	18
2.2.2 <i>Typhlodromus californicus</i>	19
2.2.3 <i>Neoseiulus californicus</i> & <i>Neoseiulus chilensis</i>	20
2.2.4 <i>Neoseiulus californicus</i> & <i>Neoseiulus fallacis</i>	21
2.3 Drawings of <i>N. californicus</i>	25
2.3.1 <i>Materials and Methods</i>	25
2.3.2 <i>Results</i>	25
2.4 The effect of preparation on spermatheca shape	31
2.4.1 <i>Materials and Methods</i>	31
2.4.2 <i>Results</i>	31
2.5 Measurements of morphological characteristics	33
2.5.1 <i>Materials and Methods</i>	33
2.5.2 <i>Results</i>	34
2.6 SEM study of chelicerae	34
2.6.1 <i>Materials and Methods</i>	34
2.6.2 <i>Results</i>	36
2.7 Discussion	41
 CHAPTER 3: OCCURRENCE OF <i>N. CALIFORNICUS</i> WORLDWIDE AND IN THE UK	 45
3.1 The worldwide occurrence of <i>N. californicus</i>: a literature review	45
3.1.1 <i>Early records</i>	45
3.1.2 <i>Europe</i>	45
3.1.3 <i>South America</i>	46
3.1.4 <i>Africa & Asia</i>	47
3.2 Occurrence of <i>N. californicus</i> in the UK	52
3.2.1 <i>Introduction</i>	52
3.2.2 <i>Materials and methods</i>	52
3.2.3 <i>Results</i>	53
3.3 Discussion	57

CHAPTER 4: WINTER SURVIVAL	61
4.1 Introduction	61
4.2 Ability to diapause	64
4.2.1 <i>Materials and methods</i>	65
4.2.2 <i>Results</i>	65
4.2.3 Discussion	66
4.3 Low temperature survival	68
4.3.1 Introduction	68
4.3.2 Supercooling point	69
4.3.2.1 <i>Materials and methods</i>	69
4.3.2.2 <i>Results</i>	71
4.3.3 Lethal Temperature	71
4.3.3.1 <i>Materials and methods</i>	71
4.3.3.2 <i>Results</i>	72
4.3.4 Lethal Time	72
4.3.4.1 <i>Materials and methods</i>	72
4.3.4.2 <i>Results</i>	73
4.3.5 Discussion	74
4.4 Field collections	78
4.4.1 Optimising the Tullgren funnel mite-extraction technique	78
4.4.1.1 <i>Materials and methods</i>	78
4.4.1.2 <i>Results</i>	81
4.4.1.2.1 <i>Hop</i>	81
4.4.1.2.2 <i>Apple</i>	82
4.4.2 Overwintering of <i>N. californicus</i> in apple, hop and blackcurrant plantations	83
4.4.2.1 <i>Materials and methods</i>	83
4.4.2.1.1 <i>Hop</i>	83
4.4.2.1.2 <i>Apple</i>	86
4.4.2.1.3 <i>Blackcurrant</i>	86
4.4.2.2 <i>Results</i>	89
4.4.2.2.1 <i>What mites were found overwintering?</i>	89
4.4.2.2.1.1 <i>Hop</i>	89
4.4.2.2.1.2 <i>Apple</i>	92
4.4.2.2.1.3 <i>Blackcurrant</i>	93
4.4.2.2.2 <i>Is there any effect of band type, variety or plot on the numbers of mites overwintering within?</i>	96
4.4.2.2.2.1 <i>Hop</i>	96
4.4.2.2.2.2 <i>Apple</i>	97
4.4.2.2.2.3 <i>Blackcurrant</i>	97
4.4.2.2.3 <i>Is there an effect of band size on numbers of mites overwintering within?</i>	98
4.4.2.2.4 <i>Were any other beneficial arthropods found sheltering in the overwintering bands?</i>	98
4.4.2.2.5 <i>What was the internal and external temperature difference in the overwintering bands?</i>	100
4.4.2.2.5.1 <i>Hop</i>	100
4.4.2.2.5.2 <i>Blackcurrant</i>	102

4.4.3 Discussion	102
4.5 Overall Discussion	104
 CHAPTER 5: POTENTIAL AS A BIOCONTROL AGENT	 105
5.1 Introduction	105
5.2 the potential of <i>N. californicus</i> as a biocontrol agent: a literature review	108
5.2.1 <i>Tetranychus urticae</i>	108
5.2.2 <i>Panonychus</i> sp.	111
5.2.3 <i>Oligonychus</i> sp.	111
5.2.4 <i>Polyphagotarsonemus latus</i>	112
5.2.5 <i>Phytonemus pallidus</i>	112
5.2.6 <i>Frankinella occidentalis</i>	113
5.3 Rearing methods	113
5.3.1 Culturing <i>N. californicus</i> and <i>T. pyri</i>	113
5.3.2 Culture plate adapted for studying individual mites	115
5.3.3 Culturing <i>T. urticae</i> and <i>P. ulmi</i>	115
5.4 Consumption rates of <i>N. californicus</i> and <i>T. pyri</i> , feeding on <i>T. urticae</i> and <i>P. ulmi</i>	116
5.4.1 Materials and methods	116
5.4.2 Results	117
5.5 Developmental rates of <i>N. californicus</i> and <i>T. pyri</i> , feeding on <i>T. urticae</i> and <i>P. ulmi</i>	118
5.5.1 Materials and methods	118
5.5.2 Results	119
5.6 Fecundity of <i>N. californicus</i> and <i>T. pyri</i> , feeding on <i>T. urticae</i> and <i>P. ulmi</i>	122
5.6.1 Materials and methods	122
5.6.2 Results	123
5.7 Discussion	123
 CHAPTER 6: STRAIN DIFFERENCES	 128
6.1 Introduction	128
6.2 Cross-mating experiment	128
6.2.1 Materials and methods	128
6.2.2 Results	129
6.3 Measurements of morphological characteristics	130
6.3.1 Materials and methods	130
6.3.2 Results	130
6.4 Developmental rates	134
6.4.1 Materials and methods	134
6.4.2 Results	135
6.5 Fecundity	136
6.5.1 Materials and methods	136
6.5.2 Results	136
6.6 Electrophoresis technique for distinguishing strain differences	137
6.6.1 Materials and methods	137
6.6.2 Results	138

6.7 Discussion		140
CHAPTER 7: FINAL DISCUSSION		145
Appendices		
I	Polyvinyl alcohol mountant Dehydration of mites for SEM	151
II	Example of a supercooling point trace obtained from the Mettler Toledo DSC820, evaluated by STAR ^e software (v6.1)	152
III	Figure II.1 Hop plot (DR149, HRI East Malling), 1998-1999. Figure II.2 Hop plot (DR149, HRI East Malling), 1999-2000.	153
IV	Figure III.3 Apple orchard, Stoke Fruit Farm, Suffolk, 1998-1999 Figure III.4 Blackcurrant plot, Upper Horton Farm, Chartham Hatch, Kent, 1998-1999	154
V	Figure IV.1 Blackcurrant plot, Rosemary Lane, Ticehurst, Kent, 1999-2000.	155
VI	Plot treatments in hop garden, HRI-EM, 1998-99	156
VII	Electrophoresis recipes	157
References		158

LIST OF TABLES

1.1 Pesticides mentioned in text arranged according type, with the concentration used	11
1.2 Response of <i>N. californicus</i> to the pesticides mentioned	13
2.1 Summary of descriptions of <i>N. californicus</i>	23
2.2 Distinguishing features of <i>N. californicus</i> and species that are similar in appearance	24
2.3 Measurements of spermatheca cervix and dorsal shield length and width, mean difference and percentage increase	33
2.4 Mean measurements of a selection of morphological characteristics of <i>N. californicus</i>	35
2.5 Previously published measurements for some morphological characteristics of adult female <i>N. californicus</i> , compared with the results of the present study	44
3.1 The chronological order of records of <i>N. californicus</i> with country, host plant and citation	48
3.2 Mites collected from dwarf hop during 1997-2000	54
3.3 Mites collected from soft fruit during 1997-2000	55
3.4 Mites collected from apple during 1997-2000	56
4.1 The critical photoperiod of a range of phytoseiid mites	65
4.2 The percentage of individuals of <i>T. pyri</i> and three strains of <i>N. californicus</i> that underwent diapause in short day conditions	66
4.3 Mean SCP values for several mite species, reared in conditions of either long daylength or short daylength	71
4.4 Percentage survival of phytoseiid mites after varying lengths of exposure at -7°C	74
4.5 Mean number of mites per band obtained from a hop garden (HRI, East Mallings) over a period of extraction in Tullgren funnels	82
4.6 Mean number of mites per band obtained from leaf litter (LL) and hop cones (C) from a hop garden (HRI, East Mallings) over a period of extraction in Tullgren funnels	82
4.7 Mean number of mites per band obtained an apple orchard (Stoke Fruit Fm, Suffolk) over a period of extraction in Tullgren funnels	83
4.8 Mites found in overwintering bands, leaf litter and cones in a hop garden (HRI, East Mallings), 1998-1999.	89
4.9 Mites found in overwintering bands, leaf litter and cones in a hop garden (HRI, East Mallings), 1999-2000.	91
4.10 Mean no. of mites obtained per band from an apple orchard (HRI, East Mallings), January 1999.	92
4.11 Mean no. of mites obtained per band from an apple orchard (Stoke Fruit Fm, Suffolk), 1998-1999.	93
4.12 Mites found in overwintering bands and leaf litter in a blackcurrant plantation (Upper Horton, Chartham Hatch), 1998-1999	94
4.13 Mites found in overwintering bands and leaf litter in a blackcurrant plantation (Rosemary Lane, Ticehurst), 1999-2000	94
4.15 The effect of band type, plot treatment, variety or row number on the quantity of phytoseiids found in overwintering refuges	98
4.16 A summary of the other beneficial arthropods found overwintering in refuges in hop, apple and blackcurrant plantations	100

5.1 Consumption rates for each life stage of <i>N. californicus</i> and <i>T. pyri</i> when fed on either <i>T. urticae</i> or <i>P. ulmi</i>	118
5.2 Developmental times for each stage of <i>N. californicus</i> and <i>T. pyri</i> when fed on either <i>T. urticae</i> or <i>P. ulmi</i>	120
5.3 Published data for mean egg to adult development times for <i>N. californicus</i>	124
5.4 Published data for mean egg to adult development times for <i>T. pyri</i>	125
6.1 Cross mating of <i>N. californicus</i> from different countries	129
6.2 Mean lengths of selected morphological characteristics for different 'strains' of <i>N. californicus</i>	131
6.3 Mean development times for <i>N. californicus</i> from different origins	135
6.4 The fecundity of different strains of <i>N. californicus</i>	137

LIST OF ILLUSTRATIONS

2.1 Dorsal surface of <i>N. californicus</i> with Chant's (1959) setal nomenclature on the left-hand side of the diagram and Lindquist and Evans' (1965) nomenclature on the right.	17
2.2 Adult female <i>N. californicus</i>	26
2.3 Male <i>N. californicus</i>	27
2.4 Larva of <i>N. californicus</i>	28
2.5 Protonymph of <i>N. californicus</i>	29
2.6 Deutonymph of <i>N. californicus</i>	30
2.7 Drawings of spermathecae from the same individual mite of two species of phytoseiid when mounted with threads under the coverslip and when flattened	32
2.8 <i>Neoseiulus californicus</i> with chelicerae extended (viewed from the side)	37
2.9 <i>Neoseiulus californicus</i> with chelicerae extended (viewed from the front)	37
2.10 <i>Neoseiulus californicus</i> gnathosoma: ventral surface	37
2.11 <i>Neoseiulus californicus</i> chelicera	38
2.12 <i>Neoseiulus californicus</i> chelicera	38
2.13 <i>Typhlodromus pyri</i> chelicerae	38
2.14 <i>Neoseiulus californicus</i> side view	39
2.15 Dorsal view of <i>N. californicus</i>	39
2.16 Leg IV of <i>N. californicus</i>	39
2.17 Ventrianal shield of <i>N. californicus</i>	40
2.18 Pores on the ventrianal shield of <i>N. californicus</i>	40
2.19 Pores on the ventrianal shield of <i>N. californicus</i>	40
3.1-3.4 Records of <i>N. californicus</i> from the 1950's to the 1990's depicted on a world map	50 & 51
3.5 Outline of the UK showing regions in which <i>N. californicus</i> has been known to be released and areas in which it was found during 1997-2000	60
4.1 Diagram of the thermal analysis system used in the 'Boersma' DTA (McNaughton & Mortimer)	70
4.2 Theoretical diagram of supercooling point distribution of a mite	75
4.3 Trellis in hop garden (HRI, East Malling), with hessian and velour overwintering bands	79
4.4 Velour overwintering band around hop bine	80
4.5 Hop cones in winter	85
4.6 Hessian overwintering band on blackcurrant stem with temperature logger	88
4.7 Mean number of mites obtained per band throughout the winter. Hop garden, HRI-East Malling, 1998-1999	90
4.8 Mean number of mites obtained per band throughout the winter. Hop garden, HRI-East Malling, 1999-2000	90
4.9 Mean number of mites obtained per band throughout the winter. Blackcurrant plantation, Rosemary Lane, Ticehurst, 1999-2000	95
4.10 The effect of refuge design on abundance and type of mites recovered from a hop garden (HRI, East Malling, 1998-99)	95
4.11 Numbers of phytoseiids obtained from overwintering bands of varying size. Hop garden, HRI, East Malling.	99
4.12 Numbers of phytoseiids obtained from overwintering bands of varying size. Apple orchard, Stoke Fruit Farm, East Anglia, 1998-99	99

4.13	Temperatures recorded over three days inside and outside an overwintering band. Readings were taken every 30 mins. Hop, HRI, East Malling, 1999-00	101
5.1	Culture plate for rearing predatory mites	114
5.2	Culture plate for studying individual mites	114
5.3	Diagram of experimental arena for studies involving predatory and phytophagous mites	117
5.4	Total mean development times from egg to adult for <i>N. californicus</i> and <i>T. pyri</i> fed on either <i>T. urticae</i> or <i>P. ulmi</i>	121
6.1	Correlation matrix and matrix scatterplot for all the samples together	132
6.2	CVA scatterplots for a combination of seven variates and a combination of shield measurements only	133
6.3	Examples of three electrophoresis gels stained for esterase activity	139
6.4	Scatterplot showing the spread of results obtained for fecundity of <i>N. californicus</i> of different origins	142

CHAPTER 1: INTRODUCTION

1.1 Introduction

Neoseiulus californicus (McGregor) belongs to the family Phytoseiidae (suborder Gamasida, super-family Phytoseioidea). Phytoseiids are free-living, terrestrial mites and most are predaceous on tetranychid mites and other small arthropods (Chant, 1985). The body of a phytoseiid is divided into two main regions; the gnathosoma (which includes the chelicerae and palps) and the idiosoma (to which the legs are attached). The idiosoma is covered by a dorsal shield, and ventrally by a number of smaller shields. There are setae on both the dorsal and ventral surfaces. The shape of the shields and the pattern of setae are the main identifying characteristics when classifying phytoseiid mites.

Neoseiulus californicus (like all phytoseiids) has five developmental stages: egg, larva, protonymph, deutonymph and adult. The larval stage has three pairs of legs; all the other motile stages have four. Setae are gained during each moult. The male is smaller than the female, has a different shaped ventrianal shield and has a spermatodactyl on each of the chelicerae. The male transfers spermatophoral material via the spermatodactyl into the spermatheca, a structure situated between the third and fourth leg of the female, during copulation.

The information available on the biology of *N. californicus* is presented below. A section is also included on the effect of pesticides on *N. californicus* as this has not been reviewed previously.

1.1.1 Biological characteristics of *N. californicus*

The mean development time from egg to adult for *N. californicus*, fed on *Tetranychus urticae* Koch, decreased from 22 days at 13°C to four days at 33°C (Castagnoli & Simoni, 1991). The threshold temperature below which development could not be completed was 8.99°C. Castagnoli and Simoni found that net reproductive rate was at a maximum at 29°C, and at this temperature the population doubled approximately every two days. Development from egg to adult required 89.98 day degrees (DD), above a threshold temperature of 8.99°C. Ma and

Laing (1973) found the total development time for *N. californicus* females, fed on *T. urticae*, ranged from 11.9 days at 16.4°C to 3.9 days at 32°C.

Neoseiulus californicus seemed to require high humidity for short development times (Castagnoli & Simoni, 1994). Castagnoli and Simoni (1994) carried out an experiment in which high temperatures (between 29°C and 30°C) and high humidities (95-100%) minimised mortality and decreased development times considerably. They found that the higher the temperature, the higher the RH needed for successful development. Egg hatching time at 21°C was over twice that at 33°C, and larval survival was significantly less at lower temperatures and humidities. The 'saturation deficit' (which is linked to relative humidity) at which 50% of eggs fail to hatch (SD₅₀) for *N. californicus* collected in Columbia was found to be high compared with *Typhlodromalus* sp., and *Phytoseiulus macropilis* (Banks), but was lower than that of *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus idaeus* Denmark & Muma (Bakker *et al.*, 1993). However, a study of humidity effects on *N. californicus* adults in the laboratory, indicated that it consumes more prey at low humidity (30%) on tomato (*Lycopersicon esculentum*), pepper (*Capsicum annum*), aubergine (*Solanun melongena*) and cucumber (*Cucumis sativus*) leaves than at high humidity (65 and 90%) (Rott & Ponsonby, 1998; Rott & Ponsonby, 2000). In Dosse's (1958a) experiments *N. californicus* tolerated 35°C and could reproduce at that temperature, but Swirski *et al.* (1970) found that at 37-40°C only a few eggs were laid and these did not hatch; no note was made of the humidity.

Castagnoli *et al.* (1995) compared the response of a laboratory strain of *N. californicus* in natural climatic conditions with those kept in the laboratory. In 1992 and 1993, from July-October, *N. californicus* were kept on artificial arenas outside, in the Tuscany region of Italy, and fed on *T. urticae* and *Quercus* sp. (oak) pollen. The egg-to-egg times were seven days at a mean temperature of 28.6°C, and 11 days at a mean temperature of 20.8°C. A higher rate of intrinsic increase was obtained in the second year (0.287 day⁻¹ compared with 0.169 day⁻¹, at 20.8°C). The degree-days (they state the threshold to be 10.71°C, however they refer to their previous paper in which the threshold was 8.99°C (Castagnoli & Simoni, 1991)) for egg-to-egg development were 133.35 in the first year, and 126.83 in the second, at 28.6°C. At 20.8°C the degree-days were 109.96 in the first year, and 93.51 in the second. When they compared these results with their data for the same strain reared under laboratory conditions

(Castagnoli & Simoni, 1991), they concluded that laboratory data on mass-reared phytoseiids are able to provide a rough estimate of the mite's attributes in the field. However, knowledge of the history and characteristics of the strain used is also necessary when predicting how effective a predatory mite will be (Castagnoli *et al.*, 1995).

The average duration of the first copulation for *N. californicus* is 351 minutes. If copulation was stopped by the observer after half this time, then there was a resulting reduction in the percentage of females that laid eggs, fecundity, oviposition duration and the percentage of females in the progeny (Castagnoli & Ligouri, 1991). Castagnoli and Ligouri (1991) found that the number and size of endospermatophores had no correlation to either the time at which oviposition commenced or the total number of eggs produced. Diet had an effect on egg production; females with curtailed copulation did not produce eggs for over a month when fed on *Carpobrotus* sp. (fig) pollen, but began to oviposit when fed on *T. urticae*. Rate of consumption of *T. urticae* eggs increased during the egg laying period of *N. californicus* (Ma & Laing, 1973).

Simoni (1992) found that in the laboratory it was possible to sex different aged eggs of *N. californicus* using an aceto-orcein temporary squash method. *Neoseiulus californicus* is a haplo-diploid species ($n=4$, $2n=8$). Simoni showed that the sex ratio in eggs of 0-2 hours old was not significantly different from that at 2-12 and 12-24 hours. This suggests that the male haploid condition occurs early in embryogenesis (Simoni, 1992). Previous phenotypic and cytogenetic research has indicated that male haploidy occurs as a result of elimination of a set of chromosomes, and for the mites studied this has occurred soon after syngamy (Schulten, 1985). Perrt-Minnot *et al.* (2000) have used co-dominant direct amplification of length polymorphism (DALP) markers to show that pseudoarrhenotoky in *N. californicus* is indeed a result of chromosome elimination in male tissues. However, they also discovered that the paternal genome is retained in males but not transmitted through sperm.

In apple (*Malus pumila*) orchards, in Chile, fertilised *N. californicus* females have a pre-ovipositional period of 4-6 days before they start laying eggs. They lay 2 eggs every 3 days during the first week, and subsequently a maximum of 2.5 eggs per day for 12 days. Given

enough food, the adults live for an average period of 18 days, and it is estimated that they have 5 generations per year (Gonzalez, 1971).

1.1.2 Effect of prey type on the biology of N. californicus

Swirski *et al.* (1970) found that *Tetranychus cinnabarinus* (Boisduval), *Eutetranychus orientalis* (Klein) and *Brevipalpus phoenicis* (Geijskes) were all suitable prey (89-100% reached maturity) for *N. californicus* and the greatest oviposition rates were achieved with *T. cinnabarinus* and *E. orientalis* (2.37 eggs/day and 1.25 eggs/day respectively). When fed the citrus rust mite, *Phyllocoptruta oleivora* (Ashmead), all the young predators died. When fed on *Polyphagotarsonemus latus* (Banks) (at 25°C, 90%RH and 16L:8D) the time taken for development from egg to adult ranged from 6 to 7 days for *N. californicus* (Castagnoli & Falchini, 1993). Juvenile mortality was very low (0.81%) and females laid a mean of two eggs per day.

Neoseiulus californicus were collected from citrus in the Mediterranean in 1971 (McMurtry, 1977) and were found to have a high ovipositional rate of 1.72 eggs per day when fed eggs and larvae of *Tetranychus pacificus* McGregor. When fed on *T. pacificus* or *T. urticae*, *N. californicus* can build up to 'unusually high densities' in culture and can reproduce on small amounts of food (McMurtry, 1977). At 25°C, 70%RH and 12L:12D photoperiod *N. californicus* had a development time from egg to adult of 107h (8.9 days) when fed on *T. urticae* and 113h (9.4 days) when fed on *Mononychellus tanajoa* Bondar (Mesa *et al.*, 1990). *Neoseiulus californicus* had a survivorship of $\geq 96\%$ on either prey species. Prey species also affected duration of oviposition period and fecundity; for *N. californicus* this was 18.1 days and 44.8 total eggs/female when fed on *T. urticae* and 15.5 days and 36.7 total eggs/female on *M. tanajoa*. De Moraes and McMurtry (1985) carried out a study comparing *Tetranychus evansi* Baker & Pritchard and *T. urticae* as prey for different phytoseiid mite species. At 25°C, 40% RH and 12L:12D photoperiod, *N. californicus* displayed an oviposition rate of two eggs/female/day when feeding on *T. urticae* and 0.5 eggs/female/day when feeding on *T. evansi*. *Neoseiulus californicus* had a greater tendency to congregate under filter paper discs treated with extracts of bean (*Vicia* sp.) leaves infested with *T. urticae*, as opposed to extracts of avocado (*Persea americana*) leaves infested with *Oligonychus punicae* (Hirst) (McMurtry *et al.*, 1991). This is consistent with observations on prey selection of *N. californicus*: it

commonly occurs on strawberry (*Fragaria* sp.) and other plants in association with *T. urticae* but has not been recorded in association with *O. punicae* on avocado (McMurtry *et al.*, 1991).

Swirski *et al.* (1970) also included potential insect prey in their study: they found that the moth larvae *Spodoptera littoralis* (Boisduval) and *Ectomyelois ceratoniae* (Zeller), tobacco whitefly (*Bemisia tabaci* (Gennadius)), Florida wax scale crawlers (*Ceroplastes floridensis* Comstock) and the thrips *Retithrips syriacus* (Mayet) were not suitable. When fed first instar citrus flower moth, *Prays citri* (Millière), approximately 67% reached maturity, but the adult females were unable to lay eggs. Crawlers of the California red scale, *Aonidiella aurantii* (Maskell) were suitable prey (89% reached maturity). The oviposition rate was low for all insect prey; *A. aurantii* gave the highest results at 0.53 eggs/day.

In addition, Swirski *et al.* (1970) examined pollen as an alternative food source. They found maize (*Zea mays*), castor bean (*Carpobrotus edulis*), almond (*Amygdalus communis*) and avocado pollens to be suitable (73-100% reaching maturity), and cotton (*Gossypium* sp.) pollen to be unsuitable (0% reaching maturity). The oviposition rate was highest for castor bean, and avocado (1.07-1.12 eggs/day), and moderate for maize and almond (0.88 and 0.81 eggs/day). Four generations of *N. californicus* were bred on castor bean pollen, and it did not affect the reproductive capacity of females under experimental conditions (Swirski *et al.*, 1970). When fed on *Malephora crocea* (iceplant) pollen the oviposition rate of *N. californicus* was lower (1.15 eggs per day) than when fed on *T. pacificus* (1.72 eggs per day) and 50% of the females were dead or missing by the end of the experiment (11 days) (McMurtry, 1977). Only six of the resulting immatures completed development and none of these females laid eggs.

Having examined the literature outlined above, it was concluded that *T. urticae* would be an appropriate food source for culturing *N. californicus* and that this could be supplemented, but not replaced by, pollen.

1.1.3 Effect of pesticides on *N. californicus*

When examining the literature concerning the effect of pesticides on *N. californicus*, it is difficult to make comparisons. Studies have been conducted in both the field and the laboratory and techniques for both differ greatly.

1.1.3.1 Acaricides

In a field trial in Argentina, the acaricide fenpyroximate was found to be particularly harmful, resulting in a 55-99% reduction of *N. californicus* during the growing season (Müther *et al.*, 1996). However, a Potter-Tower study on the effect of a lower rate of fenpyroximate (0.008%) on beneficials in orchards, in S. France, found that it was of low toxicity to *N. californicus* (Bourgouin *et al.*, 1993), causing a mortality of 6%. Bourgouin *et al.* suggest that if fenpyroximate is used in the summer when populations of *N. californicus* have established, then it would be complementary to the predator's activity (as its susceptibility to this acaricide is low), and the predators would continue to be effective after the acaricide application.

In Potter-Tower tests on mites from France, propargite caused 9% mortality of *N. californicus*, pyridaben 72% mortality and methidathion 78% mortality (Bourgouin *et al.*, 1993). However, Costa-Comelles *et al.* (1994a) in field trials in Spain, found propargite to cause population reductions of over 50%.

The acaricides dicofol and dicofol + tetradifon caused 75-98% mortality in Potter-Tower tests on mites from S. France, whereas cyhexatin produced 38% mortality, though results were variable between different populations (Fauvel & Bourgouin, 1993).

In field trials in Spain, amitraz, benzoximate, dinobuton, fenazaquin and pyridaben caused population reductions of over 50% (Costa-Comelles *et al.*, 1994a).

Potter-Tower tests, carried out in France and Spain, found acrinathrin to be selective to *N. californicus*; it was found to give 0% mortality compared to methidathion for which the mortality was 43-86% (Mattioda *et al.*, 1990; Heller *et al.*, 1992). Heller *et al.* recommend rational use of acrinathrin for tetranychid control, to avoid the development of resistance.

Neoseiulus californicus is able to survive acarathrin and maintain tetranychid mites under an acceptable threshold (Mattioda *et al.*, 1990). In a field study in Argentina, Giganti (1993) found acarathrin to have no harmful effect on *N. californicus*.

The effects of some acaricides on *N. californicus* were examined in field trials in Chile. Soil treatment with carbofuran and foliar applications of mevinphos and pirimicarb were effective at controlling *T. urticae*. The soil applications of carbofuran did not harm *N. californicus* populations, nor did treatments with pirimicarb or oxydemeton-methyl. However, mevinphos was not as selective and caused fluctuations in the predator population (Arretz *et al.*, 1976). In Uruguayan apple orchards, *N. californicus* was highly resistant to fenbutatin (Bruhn & Beltrame, 1981).

A range of acaricides was tested on *T. urticae* and *N. californicus* (from Hawaii), in a Potter-Tower by Wu *et al.* (1985). Bromopropylate, dinobuton and propargite allowed greatest survival of the phytoseiid, whilst affording effective control of the two-spotted spider mite. Formetanate and dinobuton were the most effective against the two-spotted spider mite. *Tetranychus urticae* rapidly developed resistance to formetanate whereas it developed resistance to dinobuton only after 15-16 generation selections. Dinobuton would, therefore, be the most appropriate acaricide to use in an IPM programme. *Neoseiulus californicus* was sensitive to the organophosphorous compounds tested, having an LC₅₀ of <0.07mg/ml (ethion, prothoate, dialifor, dimethoate and omethoate), and synthetic pyrethroids, with an LC₅₀ of <1.9x10⁻³mg/ml (permethrin, decamethrin, cypermethrin, fenpropathrin, flucythrinate and fenvalerate) (Wu *et al.*, 1985).

1.1.3.2 Insecticides

Steiner and Elliott (1983) summarised the reported toxicity of common greenhouse pesticides on various biological control agents, including *N. californicus*. Permethrin, carbaryl, dicofol and cyhexatin were highly toxic to one or more stages of the mite. However, no country of origin of the mites or application rates were given.

Gonzalez (1971) reported that Chilean *N. californicus* were susceptible, in the field, to phosphates, dinitros, iron compounds (e.g. cyhexatin) and carbamates. They had a greater

tolerance to certain chlorate products, sulphates and endosulphans, though the populations were reduced to very low levels. Winter oil treatments did not eliminate overwintering females, but if combined with phosphates, dinitros or dinitrobutylphenol-substitutes a high mortality resulted (Gonzalez, 1971). However, no application rates were given.

In Potter-Tower tests using mites from S. France, fluvinate and vamidothion and phosmet were found to be relatively 'safe' (5-15% mortality), while phosalone was toxic (65-70% mortality) and carbaryl was of moderate toxicity (65% mortality) to *N. californicus* (Fauvel & Bourgouin, 1993).

Two insecticides were tested for fruit fly (*Anastrepha fraterculus* (Wiedemann)) control in Brazilian apple orchards and their effects on *Panonychus ulmi* (Koch) and *N. californicus* were also examined. In the plots treated with phosmet large numbers of *N. californicus* were observed and *P. ulmi* was satisfactorily controlled. However, on the plots treated with fenthion few predators were observed and two acaricide applications were required to control *P. ulmi*. The substitution of phosmet in place of fenthion for control of *A. fraterculus* would be compatible with biological control of *P. ulmi* without compromising fruit quality as it is equally effective against fruit fly (Monteiro, 1993). No application rates were given.

In orchards in the Dordogne region of France, which had been managed with an IPM programme, it was found that there were three prevalent phytoseiid species, *Amblyseius andersoni* (Chant), *N. californicus* and *Euseius finlandicus* (Oudemans) (in descending order of abundance) (Baudry & Reigne, 1993). The effect of insecticides, applied against codling moth, on these predatory mites was examined. On plots treated with fluvalinate, *N. californicus* replaced *A. andersoni* as the most abundant phytoseiid. Fenoxycarb seemed to have an effect on phytoseiid reproduction and Potter tower tests showed that fluvalinate was toxic to *A. andersoni* and phosalone was toxic to *N. californicus* (Baudry & Reigne, 1993).

The effect of various pesticides on *N. californicus* was tested in field trials, in Spain. Fluvalinate, phosalone, methidathion, and pirimiphos-methyl all caused population reductions of up to 75%. Diazinon, chlorpyrifos-ethyl, chlorpyrifos-methyl, hexaflumuron. mecarbam

and azinphos-methyl caused reductions of up to 50%, and diflubenzuron, fenoxycarb, dimethoate and phosmet <25% (Costa-Comelles *et al.*, 1994a).

Croft *et al.* (1976) found populations of *N. californicus* in Uruguayan apple orchards that had developed resistance to organophosphorus insecticides. In particular, almost all *N. californicus* survived when phosmet was applied, and many survived when azinphos-methyl was used. Carbaryl and azinphos-ethyl, used to control lepidopteran larvae in Uruguay, had no adverse effect on *N. californicus* (Bruhn & Beltrame, 1981).

Bacillus thuringiensis was found to cause a population reduction of <25%, in field trials in Spain (Costa-Comelles *et al.*, 1994a).

1.1.3.3 Fungicides

Benzimidazole and maneb were reported to be highly toxic to one or more stages of *N. californicus*, whereas streptomycin and chlorothalnil were reported to be of negligible toxicity (Steiner & Elliott, 1983). However no application rates or country of origin of the mites were given.

In Uruguay, the acaricidal properties of some fungicides applied in springtime (in particular propineb, maneb and mancozeb that are used for control of apple scab (*Venturia inaequalis*), can reduce numbers of *P. ulmi*, without harming *N. californicus*. Captan, dodine and thiram were found to have no effect on either phytophagous or predatory mites. Benzimidazoles had a variable effect on both phytophagous and predatory mites (Bruhn & Beltrame, 1981). No application rates were given.

Table 1.1 shows the pesticides mentioned in the literature by type, and the application rates used. The application rates have been converted into parts per million (ppm) to enable easier comparison. Table 1.2 summarises the effect of the various pesticides on *N. californicus* in relation to its country of origin.

In summary, of the pesticides tested, *N. californicus* was susceptible to most pyrethroids (except acrinathrin, fluvalinate and fluvinate) and about half the organophosphates. It was

mostly tolerant to the carbamate, organotin and benzoyl-urea compounds tested and susceptible to the organochlorines. There does not appear to be a distinct relationship between country of origin and pesticide susceptibility. In fact, comparisons are difficult as different pesticides were used, under different conditions by different authors.

Table 1.1 Pesticides mentioned in the text arranged according to type, with the concentration used. The right-hand column indicates the response of *N. californicus* to the pesticide: t = tolerant or resistant, <25% mortality; m = moderately susceptible, approx 50% mortality; s = susceptible, >50% mortality.

* are field trials; where no concentration is given field rates were presumably used.

Type	Pesticide	concentration	
organophosphorus insecticide (anti-cholinesterases – cholinesterases are chemical transmitters in nerve synapses, therefore these chemicals act as central nerve poisons)	azinphos-ethyl	none given (Bruhn & Beltrame, 1981) *	t
	azinphos-methyl	400 ppm (Costa-Comelles <i>et al.</i> , 1994a)	m
	chlorpyrifos-ethyl	1000 ppm (Costa-Comelles <i>et al.</i> , 1994a)	m
	chlorpyrifos-methyl	900 ppm (Costa-Comelles <i>et al.</i> , 1994a)	m
	dialifor	680 ppm (Wu <i>et al.</i> , 1985)	s
	diazinon	1200 ppm (Costa-Comelles <i>et al.</i> , 1994a)	m
	dimethoate	140 ppm (Wu <i>et al.</i> , 1985)	s
		600 ppm (Costa-Comelles <i>et al.</i> , 1994a)	t
	ethion	120 ppm (Wu <i>et al.</i> , 1985)	s
	fenthion	none given (Monteiro, 1993) *	s
	mecarbam	1000ppm (Costa-Comelles <i>et al.</i> , 1994a)	m
	methidathion	300 ppm (Bourgouin <i>et al.</i> , 1993; Heller <i>et al.</i> , 1992; Mattioda <i>et al.</i> , 1990)	s
		600ppm (Costa-Comelles <i>et al.</i> , 1994a)	s
	mevinphos	none given (Arretz <i>et al.</i> , 1976) *	m
	omethoate	17 ppm (Wu <i>et al.</i> , 1985)	s
	oxydemeton-methyl	none given (Arretz <i>et al.</i> , 1976) *	t
	phosalone	600 ppm (Fauvel & Bourgouin, 1993; Baudry & Reigne, 1993)	s
		700ppm (Costa-Comelles <i>et al.</i> , 1994a)	s
	phosmet	500 ppm (Fauvel & Bourgouin, 1993)	t
		750 ppm (Costa-Comelles <i>et al.</i> , 1994a)	t
carbamate	pirimiphos-methyl	1000ppm (Costa-Comelles <i>et al.</i> , 1994a)	s
	prothoate	60 ppm (Wu <i>et al.</i> , 1985)	s
	vamidothion	500 ppm (Fauvel & Bourgouin, 1993)	t
	carbaryl	850 ppm (Fauvel & Bourgouin, 1993)	m
		none given (Bruhn & Beltrame, 1981) *	t
(anti-cholinesterases – cholinesterases are chemical transmitters in nerve synapses, therefore these chemicals act as central nerve poisons)	carbofuran	none given (Arretz <i>et al.</i> , 1976) *	t
	pirimicarb	none given (Arretz <i>et al.</i> , 1976) *	t
	fenoxycarb	75 ppm (Baudry & Reigne, 1993)	s
	(non neurotoxic IGR)	250 ppm (Costa-Comelles <i>et al.</i> , 1994a)	t
pyrethroid (pyrethroids target the ganglia of the central nervous system, affecting ion permeability of the axons and resulting in a suppression of nerve activity)	acrinathrin	37.5 ppm, 75 ppm, 150 ppm (Heller <i>et al.</i> , 1992; Mattioda <i>et al.</i> , 1990)	t
		16.6 ml/hl (Giganti, 1993) *	t
	cypermethrin	3.4 ppm (Wu <i>et al.</i> , 1985)	s
	fluvalinate	144 ppm (Baudry & Reigne, 1993)	t
		290 ppm (Costa-Comelles <i>et al.</i> , 1994a)	s
	decamethrin	1 ppm (Wu <i>et al.</i> , 1985)	s
	fenpropathrin	0.98 ppm (Wu <i>et al.</i> , 1985)	s
	fenvalerate	1.9 ppm (Wu <i>et al.</i> , 1985)	s
	flucythrinate	19 ppm (Wu <i>et al.</i> , 1985)	s
	fluvinate	146 ppm (Fauvel & Bourgouin, 1993)	t

	permethrin	1.5 ppm (Wu <i>et al.</i> , 1985)	S
organochlorine	dicofol	500 ppm (Fauvel & Bourgoïn, 1993)	S
(chlorinated hydrocarbon insecticides - affect ion permeability of axons, hence are nerve poisons)	dicofol + tetradifon	425 ppm + 160 ppm (Fauvel & Bourgoïn, 1993)	S
mitochondrial electron transport inhibitor	fenazaquin	200ppm (Costa-Comelles <i>et al.</i> , 1994a)	S
	fenpyroximate	750 ppm (Müther <i>et al.</i> , 1996)	S
		80 ppm (Bourgoïn <i>et al.</i> , 1993)	t
organotin	cyhexatin	300 ppm (Fauvel & Bourgoïn, 1993)	m
	fenbutatin	none given (Bruhn & Beltrame, 1981) *	t
benzoyl-urea (inhibits chitin synthesis)	diflubenzuron	130 ppm (Costa-Comelles <i>et al.</i> , 1994a)	t
	hexaflumuron	130 ppm (Costa-Comelles <i>et al.</i> , 1994a)	m
amidine	amitraz	600 ppm (Costa-Comelles <i>et al.</i> , 1994a)	S
benzilate	bromopropylate	3300 ppm (Wu <i>et al.</i> , 1985)	t
other acaricides	benzoxymate	400 ppm (Costa-Comelles <i>et al.</i> , 1994a)	S
	dinobuton	800 ppm (Costa-Comelles <i>et al.</i> , 1994a)	S
	propargite	1200 ppm (Bourgoïn <i>et al.</i> , 1993)	t
		55700 ppm (Wu <i>et al.</i> , 1985)	t
	pyridaben	150 ppm (Bourgoïn <i>et al.</i> , 1993)	S
		100ppm, 300ppm (Costa-Comelles <i>et al.</i> , 1994a)	S

Table 1.2 Response of *N. californicus* to the pesticides mentioned: t = tolerant or resistant, <25% mortality; m = moderately susceptible, approx 50% mortality; s = susceptible, >50% mortality.

Country of origin of <i>N. californicus</i>	organophosphorus insecticide	carbamate	pyrethroid	organochlorine	mitochondrial electron transport inhibitor	organotin	benzoyl-urea	amidine	benzilate	other acaricides	Author
Chile	s	s									Gonzalez (1971)
Chile	m & t	t									Arretz <i>et al.</i> (1976)
Uruguay	t										Croft <i>et al.</i> (1976)
Uruguay	t	t				t					Bruhn & Beltrame (1981)
Brazil	t & s										Monteiro (1993)
Argentina			t								Giganti (1993)
Argentina					s						Müther <i>et al.</i> (1996)
country not stated (possibly Canada)		s	s	s		s					Steiner & Elliott (1983)
Hawaii	s		s						t	t	Wu <i>et al.</i> (1985)
S. France	s				t					t & s	Bourgouin <i>et al.</i> (1993)
S. France	t & s	m	t	s		m					Fauvel & Bourgouin (1993)
France	s	s	t								Baudry & Reigne (1993)
France/Spain	s		t								Heller <i>et al.</i> (1992); Mattioda <i>et al.</i> (1990)
Spain	s, m & t	t	s		s		m & t	s		s	Costa-Comelles <i>et al.</i> (1994a)

1.1.4 The potential role of *N. californicus* as a biocontrol agent of *P. ulmi*

Neoseiulus californicus was the dominant predator of *P. ulmi* in Navarre, Spain (Iraola *et al.*, 1994). It was one of the most common phytoseiid species on both apple trees and the weeds underneath, in non-commercial orchards in Spain (Costa-Comelles *et al.*, 1994b). In Spanish peach orchards, that were treated in a selective way to control pests, the phytoseiid mite fauna showed a greater diversity and appeared earlier in the year. During the summer the phytoseiids reached levels of 1-2 mites per leaf, and the most abundant phytoseiid was *N. californicus*. However, in orchards treated with an IPM programme it was overtaken by *Amblyseius potentillae* (Garman) as the prevalent predatory mite (Villaronga *et al.*, 1993).

In the Rio Negro province of Argentina, *N. californicus* was the main predator of *P. ulmi* and was found in all orchards sampled over two years (Müther *et al.*, 1996). In most cases satisfactory control of *P. ulmi* did not occur, probably due to the delayed appearance of the predatory mite in spring. This could be caused by intensive chemical control of mites and other orchard pests or an absence of overwintering sites or alternative winter-food sources (Müther *et al.*, 1996). In a plot where pyrethroids were applied regularly up to the sampling year, the numbers of *P. ulmi* increased to 18 mites/leaf even though *N. californicus* was present. Leaf and beat samples showed a disturbed ecosystem with low density and diversity of arthropods. However, in an orchard with organic management the spider mite population was easily maintained at two mites per leaf, eriophyid and tydeid mites were present and in the following spring *N. californicus* appeared early (Müther *et al.*, 1996). *Panonychus ulmi* and *T. urticae* are important pests in apple orchards in Buenos Aires, and are associated with *N. californicus* (Monetti, 1995). Experiments carried out in an apple orchard in Argentina from November 1990 to March 1993 showed that periods of multiple pesticide application resulted in decreases in *N. californicus* populations. During the months that no pesticides were used there was a greater abundance of *N. californicus* and a decrease in the *P. ulmi* population (Monetti & Fernandez, 1995).

In a comparison of predation by *N. californicus* and *Neoseiulus fallacis* (Garman) in the laboratory, both phytoseiids had high survival rates on *P. ulmi* and reproduction (cumulative means of eggs of active immatures produced) was greater for *N. californicus* (Croft *et al.*, 1998a).

As *N. californicus* was found in previous years in the UK in apple orchards (Solomon & Fitzgerald, unpublished data), and the literature indicated that it has potential as a biocontrol agent of *P. ulmi*, the aims of this study were to establish whether *N. californicus* could be exploited as a biocontrol agent for this pest mite. This would involve

- determining whether it has established in the UK
- its overwintering potential
- its ability to survive and reproduce when feeding on *P. ulmi*

CHAPTER 2: TAXONOMY

2.1 Introduction

Taxonomy of phytoseiid mites is difficult. The few keys available are not comprehensive or have misleading descriptions. For the UK there is no specific key for phytoseiids and several keys have to be cross-referenced when identifying mites.

Most descriptions of phytoseiids are of adults; immatures are not easily identifiable as they gain setae with each moult. There is a range of morphological characters used to distinguish between mite species. These include: number and position of setae on the dorsal and ventral shields, shape of the female spermatheca, setation of the legs (in particular presence of macrosetae) and dentition of the chelicerae. There have been several different approaches to setal nomenclature, resulting in different numbering systems. For example, Chant (1959), based on Garman's (1948) system, numbered the lateral setae (L_1 - L_n) from front to back, the dorsal setae (D_1 - D_n) and the median setae (M_1 and M_2). The limitation of this numbering system is that it does not give any information about which setae are 'missing' for a particular mite species. Lindquist and Evans (1965) developed a system based on the three longitudinal rows of setae, and divided them further into anterior series and posterior series: the lateral setae (s-S), the medio-lateral setae (z-Z) and the dorso-central setae (j-J). Figure 2.1 shows the dorsal surface of *Neoseiulus californicus* with both the numbering systems applied to the setae.

Neoseiulus californicus has a complicated taxonomy. It has been described by different authors under different synonyms and has also been synonymised with at least one species incorrectly. A literature survey to elucidate the taxonomic history of *N. californicus* was carried out and a review is presented here.

The aim of the work in this chapter was to clarify the synonymies of *N. californicus*, produce definitive drawings of all stages of *N. californicus* and undertake a detailed examination of some of the morphological characteristics used to identify this mite. This should produce some clarification for the confusion that exists within the literature and provide confirmation of the existing descriptions.

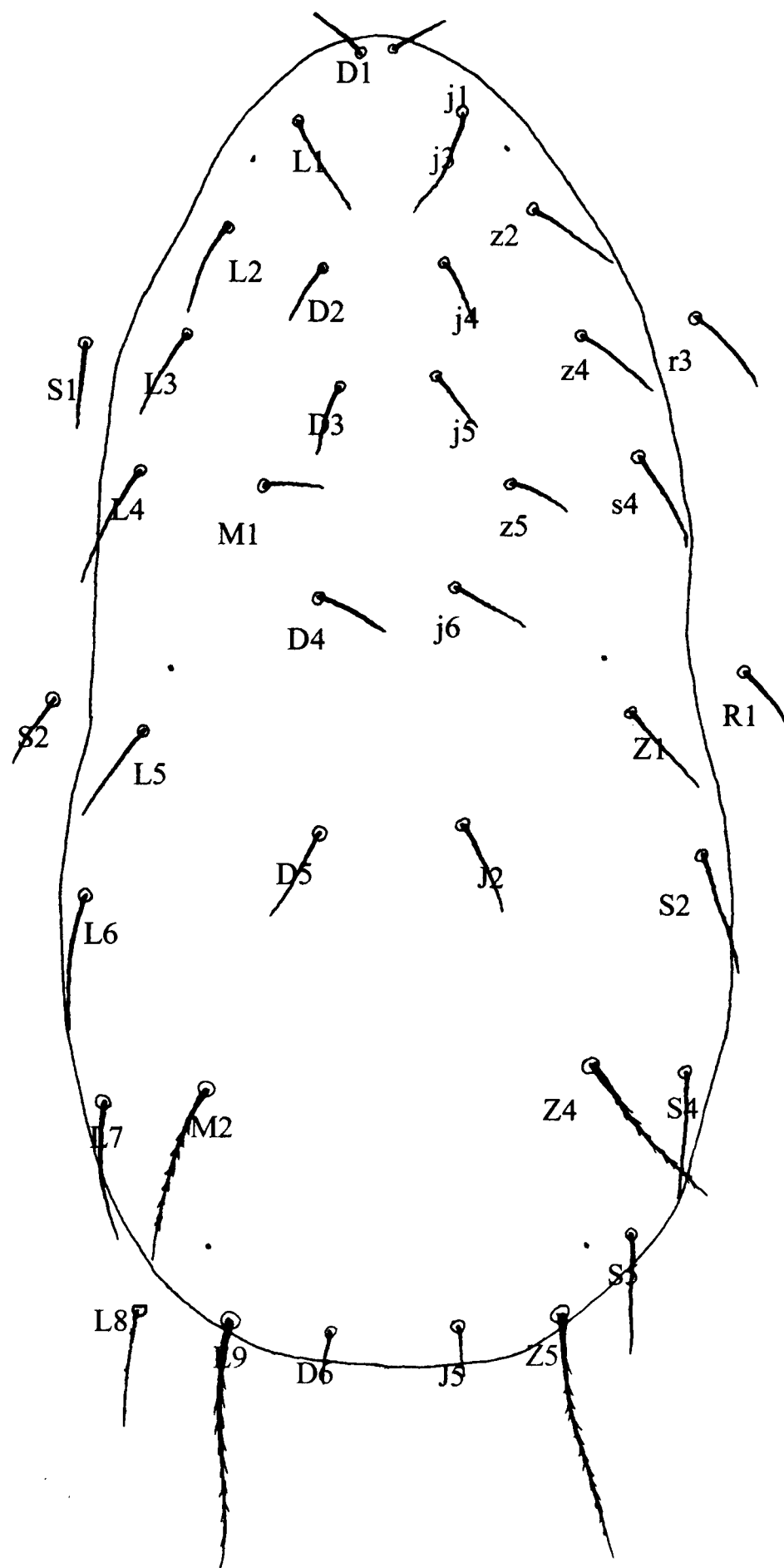


Figure 2.1 Dorsal surface of *N. californicus* with Chant's (1959) setal nomenclature on the left-hand side of the diagram and Lindquist and Evans' (1965) nomenclature on the right.

2.2 Review of the taxonomic history of *N. californicus*

Throughout this review the setal nomenclature given is that of Lindquist and Evans (1965), with the Chant (1959) nomenclature given in parentheses afterwards.

2.2.1 *Typhlodromus mungeri*

McGregor (1954) described *Typhlodromus mungeri* as a new species, from a single female found on lemon fruit in Whittier, California in February, 1953, feeding on *Panonychus citri* (McGregor). There were 17 pairs of setae on the dorsal shield, the longest being Z5 (L₉). There were three pairs of pre-anal setae and between the posterior pair were a pair of narrowly separated pores. The biting portion of the fixed chelicera had three terminally situated teeth and a long, thin spine (the pilus dentilis). The movable part had one inconspicuous tooth opposite the spine. There was a macroseta on the tarsus of leg IV. *Typhlodromus californicus* was also described as a new species by McGregor (1954). The description was based on a single male found at the same Californian site in January, 1953. There were 18 pairs of setae on the dorsal shield, the longest being Z5 (L₉). The ventrianal shield had four pairs of pre-anal setae in two transverse rows, with the anterior row being remote from the front margin of the shield. A pair of lunate pores were present between and behind the bases of the middle setae of the posterior row of pre-anals. There was a long, strong hair (macroseta) on the tarsus of leg IV, and the chelicerae were not in a position to be studied.

Chant (1959) proposed a new synonymy between *Typhlodromus marinus* Chant, *T. californicus* McGregor and *T. mungeri* McGregor. He examined McGregor's type specimens and concluded that his *T. californicus* and *T. mungeri* were synonymous. Chant proposed that McGregor's *T. californicus* male was the same as his own *T. marinus* male. The females of *T. mungeri* and *T. marinus* differed only in the shape of their 'coxal glands' (spermathecae), and Chant wondered if this was an artefact of mounting. Evans (1987) described the spermathecae of *Neoseiulus marinus* as having an 'inverted V-shaped' calyx; this differs from the 'cup' or 'bell-shaped' spermathecae of *N. californicus*. The dorsal shield of Chant's *T. marinus* (♀) had 17 pairs of setae, and nine in the lateral rows. All the setae were short, with the length of j3 and z2, z4 and s4 (L₁-L₄) being less than half the distance between their bases. It had three pairs of pre-anal setae and a pair of pores. There were four pairs of setae on the

membrane surrounding the ventrianal shield, two pairs of metapodal plates, and a macroseta on leg IV. The male's ventrianal shield had five pairs of pre-anal setae. Chant's drawing of the ventrianal shield of *T. marinus* shows no pores (Chant, 1958). *Typhlodromus marinus* was found on seaweed in the North Sea and the Thames Estuary, England, apple in West Virginia and strawberries in West Canada. It has also been found on wheat, pine, mallow, citrus, alfalfa and poplar in SW USA and lemon fruit in Morocco (Chant, 1958). Chant states that these populations are 'morphologically nearly identical'. However, differences between *T. marinus* and *T. mungeri* (length of setae, number of pre-anals and lack of pores on the ventrianal shield in the male and spermatheca shape) are sufficient to lend doubt to this synonymy. Schuster and Pritchard (1963) note that females of *A. californicus* collected in California bore no close resemblance to Chant's *T. marinus*.

2.2.2 *Typhlodromus californicus*

Athias-Henriot (1959) noted that McGregor's male *T. californicus* and female *T. mungeri* were collected from the same place and shared a number of characteristics (dorsal chaetotaxy, length of dorsal setae, macroseta on leg IV). However, she pointed out that the original descriptions do not allow definitive synonymy; in particular, no measurements of dimensions were made. She proposed *Amblyseius mungeri* as synonymous with *T. mungeri* McGregor. *Amblyseius mungeri* were collected from Algeria on *Cynodon dactylon* (bermuda grass) and France on cultivated strawberries, leaf litter, *Phaseolus coccineus* (runner bean), *Plantago major* (greater plantain), *Paspalum distichum* (joint grass), *Dactylis glomerata* (cock's foot, grass family) and *Potentilla reptans* (creeping cinquefoil, rose family).

A new combination was proposed by Schuster and Pritchard (1963) of *A. californicus*, in synonymy with *T. californicus* McGregor, *T. mungeri* McGregor, *A. mungeri* Athias-Henriot and *T. (A) marinus* (Willman) Chant. *Amblyseius californicus* was collected in Riverside and San Diego counties (California) from citrus and pecan litter, in 1958. Their description was more comprehensive than any previously made. The female's dorsal shield was 355µm long and 170µm wide and the ventrianal shield was 115µm long and 98µm wide. There were three pairs of pre-anal setae and a pair of crescentic pores medially caudal to the third pair of setae. There were two subapical teeth on the fixed digit and one on the moveable digit. The cervix of the spermatheca was cup-shaped. There was a macroseta on the basitarsus of leg IV, which

was 48µm long. The male had a ventrianal plate with three or four pairs of pre-anal setae and a pair of crescentic pores (Schuster & Pritchard, 1963).

2.2.3 *Neoseiulus californicus* & *Neoseiulus chilensis*

Muma (1961) broadly defined some characteristics of phytoseiid groups: *Amblyseius* Berlese species had three macrosetae on the fourth leg, *Cydnodromus* species had 0-1 macrosetae and *Neoseiulus* species had none. *Typhlodromus* species had four pairs of pre-anal setae and one macroseta on leg IV. *Neoseiulus californicus* has historically been attributed to each of these groups; *Neoseiulus californicus* has one macroseta on leg IV. However, this is the current nomenclature that is commonly adopted and bears no relation to Muma's definitions.

Typhlodromus chilensis was described as a new species by Dosse (1958a), on *Eichhornia crassipes* (water hyacinth) from a glasshouse. Originating from Chile, his specimens had large distinctive pores and the spermathecae were bell-shaped (similar to *A. californicus*). Athias-Henriot (1977) redefined the genus *Cydnodromus*, with *T. californicus* as the type species. The chelicerae had 2-7 teeth on the fixed digit, and 0-3 teeth on the mobile digit. A macroseta was present on leg IV. *Cydnodromus californicus* was morphologically indistinguishable from *Cydnodromus chilensis*, with a presumed synonymy with *Typhlodromus chilensis*, and has been collected from California, Chile, Maghreb (Africa) and the southern region of Europe (Athias-Henriot, 1977). Cross-breeding experiments, between Californian populations of *Neoseiulus californicus* and populations of *Neoseiulus chilensis* from either Chile or Peru, resulted in male and female progeny, supporting previous opinions of synonymy (McMurtry and Badii, 1989). Examination of specimens showed no consistent differences in dorsal shield setal measurements, spermatheca shape, ventrianal shield characteristics and leg macroseta. The absence of reproductive barriers between separated populations of *N. californicus* suggests either a recent movement to geographical areas or a relative stability of morphological and/or physical characteristics related to mating and reproduction (McMurtry and Badii, 1989). They note that this is not true for the Phytoseiidae in general; studies on *Euseius* sp. have indicated the existence of numerous strains with varying degrees of reproductive incompatibility.

Amblyseius californicus collected from citrus in Spain appeared identical to Californian specimens which had been collected from citrus and strawberries (McMurtry, 1977). *Amblyseius californicus* specimens from southern Europe, Algeria and California are indistinguishable from *Amblyseius chilensis* from Chile (McMurtry, 1977 - by personal communication with Athias-Henriot). Specimens from Guatemala, Peru and Argentina were examined and no 'consistent differences' between them and specimens from Spain or California were observed. Female *A. californicus* collected in Peru, from peach and *Manihot ultissima*, showed slight differences in the length of some dorsal setae (the particular setae were not noted). However, there were no significant morphological differences in any other characters (El-Banhawy, 1976).

There have been several more-recent descriptions of *A. californicus*. Ueckermann and Loots (1988) noted that the pre-anal pores were closely spaced and the para-anals were close to the anterior margin of the anal opening, the major duct of the spermatheca long and slender, the atrium a small bulb and the cervix bell-shaped. Kreiter and De La Bourdonnaye (1993) described *A. californicus* as having elongated Z4 (M₂) and Z5 (L₉) setae on the dorsal shield. The ventrianal shield had 3 pairs of pre-anal setae and 2 'eye-shaped' pores. They noted its synonymy with *T. californicus*, *C. californicus*, *C. chilensis*, *N. chilensis* and *N. californicus*. Çobanoğlu (1993) described specimens found on apple in Turkey; his description supports previous ones.

An SEM study on the cheliceral morphology of some Phytoseiidae, discovered that most species have a flange-like expansion on the abaxial surface of the fixed digit or 'lobe' (Flechtman & McMurtry, 1992). These lobes are often not visible with light microscopy and are soft and delicate. *Neoseiulus californicus* had a small lobe on the fixed digit, which had five teeth, two of these were basal to the pilus dentilis which was long and 'needle-like'. The movable digit had three teeth and was 'strongly hooked'.

2.2.4 *Neoseiulus californicus* & *Neoseiulus fallacis*

Neoseiulus fallacis (Garman) is morphologically very similar to *N. californicus*, differing mainly in the length of anterior lateral setae and the shape of the spermatheca. Cross-breeding experiments between *N. californicus* and *N. fallacis* have showed that they are

separate species, but that their males were promiscuous at tending and mating (Monetti & Croft, 1997). However, as mentioned above, McMurtry and Badii (1989) noted that *Euseius* sp. have numerous strains with varying degrees of reproductive incompatibility. Therefore, it is possible that *N. fallacis* is the same species and it has become reproductively isolated from *N. californicus*.

Molecular techniques for species discrimination are being developed for phytoseiid mites. Navajas *et al.* (1999) assessed some of the different markers available for six species of phytoseiid, including *N. californicus*. They found that the 5.8S gene displayed little variation between genera whereas ITS1 and ITS2 showed some differences; ITS1 was the most variable. These are found on the ribosomal RNA, ITS are Internal non-transcribed Spacer regions and ITS1 and ITS2 are either side of the 5.8S gene. *N. californicus* from France were compared with *N. californicus* from Greece and no differences were found in any of the sequences. However, differences were discovered in the ITS1 sequences with intraspecific comparisons of *N. fallacis* and *Euseius concordis* Chant. *Neoseiulus fallacis* and *N. californicus* showed a high degree of homology. The 5.8S and ITS2 regions were identical and 98.6% of neucleotides were the same for the ITS1 region (Navajas *et al.*, 1999). The most promising markers being developed for inter-specific comparisons of *N. californicus* are micro-satellites (Navajas – personal communication). These are highly repeated DNA sequences, often tandem repeats, which may have association with centromere or telomere function in insects (Hoy, 1994). This DNA evolves at a very high rate and is usually species specific (Hoy, 1994).

Table 2.1 summarises the descriptions of *N. californicus* made by different authors. It can be observed that the descriptions of *N. californicus* are, in some instances, contradictory and that there are gaps in the information. Table 2.2 shows the distinguishing features of *N. californicus* and species that are similar in appearance, and thus might be confused with it.

Table 2.1 Summary of descriptions of *N. californicus* from the literature; this shows the discrepancies and agreed characteristics.






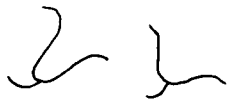




	<i>A. chilensis</i> (Gonzalez & Schuster, 1962)	<i>T. californicus</i> (McGregor, 1954)	<i>T. mungeri</i> (McGregor, 1954)	<i>C. californicus</i> = <i>T. chilensis</i> (Athias-Henriot, 1977)	<i>A. californicus</i> (Schuster & Pritchard, 1963)	<i>A. chilensis</i> (Ueckermann & Loots, 1988)
♀						
dorsal shield	reticulated, Z4 (M ₂) & Z5 (L ₉) slightly serrated	(male only)	17 pairs setae, longest Z5, 2 setae on interscutal membrane	reticulated, seta J2 (D ₃) present, Z5 and Z4 serrated	faintly reticulated	strongly reticulated, Z4 & Z5 longest and serrated
sternal shield	slightly reticulated, 3 pairs of setae	--	3 pairs setae	--	3 pairs setae, metasternal plates small but distinct	--
ventrianal shield	reticulated, pair of prominent pores (crescentic in diagram)	--	3 pairs preanals, pair of pores (long and curved from diagram)	3 pairs preanals, pair crescent shaped pores	3 pairs preanals, pair crescent shaped pores	preanal pores closely spaced
spermathecae	bell shaped with a small atrium 	--	none shown	bell-shaped: 	cup shaped: 	bell shaped: 
chelicerae						
fixed digit:	2 subapical teeth	--	3 terminally	2 to 7	2 subapical	--
moveable digit:	3 teeth	--	1	0 to 3	1	--
No. of macrosetae on leg IV	one	--	one	one	one	one
♂						
dorsal shield	--	18 pairs of setae, longest Z5. 3 small submarginal 'platelets', (one heart-shaped behind Z5)	--	--	--	--
ventrianal shield	reticulated, pair of pores (crescentic in diagram)	4 pairs preanals in 2 transverse rows, lunate pores.	--	--	3 or 4 preanals, pair crescentic pores	--
No. of macrosetae on leg IV	--	one	--	--	--	--

Table 2.2 Distinguishing features of *N. californicus* and species that are similar in appearance.

	<i>N. californicus</i> (Gonzalez & Schuster, 1962; McGregor, 1954; Athias- Henriot, 1977; Schuster & Pritchard, 1963)	<i>N. fallacis</i> (Collyer, 1982; Nesbitt 1951; Schuster & Pritchard, 1963; Athias- Henriot 1977)	<i>T. marinus</i> (Chant, 1959; Evans, 1987)	<i>A. reductus</i> (Miedema, 1987)	<i>T. umbraticus</i> (Chant, 1956; 1959; Chant & Yoshida- Shaul, 1982)	<i>A. collegae</i> (Chant & Yoshida-Shaul, 1982)
dorsal shield	reticulated, anterior lateral setae do not reach base of next one, Z4 (M ₂) & Z5 (L ₉) serrated	reticulated, anterior lateral setae reach bases of next seta, Z4 & Z5 slightly serrated	reticulated, all setae short & smooth except a few serrations on Z5	reticulated, anterior lateral setae reach bases of next seta, only Z5 slightly serrated	lightly reticulated, anterior lateral setae reach bases of next seta, Z4 & Z5 slightly serrated	lightly reticulated, Z4 & Z5 slightly serrated
sternal shield	slightly reticulated, 3 pairs of setae	3 pairs of setae, sometimes posterior pair not on shield but on a separate plate	3 pairs setae	--	3 pairs setae, metasternal setae on platelets	weakly sclerotised and not discernable
ventrianal shield	reticulated, pair of crescentic pores	pair crescentic pores	pores conspicuous, smaller than <i>A.</i> <i>californicus</i>	eye-shaped pores	lightly reticulated with a distinct waist, pair circular pores	lightly reticulated with a distinct waist, pair large crescentic pores
♀ 24 spermathecae	bell/cup shaped: 	bell shaped: 	inverted V-shaped calyx with a distinct neck: 	bell-shaped: 	cup shaped: 	bell shaped: 
chelicerae						
fixed digit:	5 teeth	1	4 or 5	--	5 or 7 or 3	6
moveable digit:	3 teeth	0	0	--	2	1
No. of macrosetae on leg IV	one	one or three	one	one	three	one
ventrianal shield	reticulated, pair of crescentic pores	3 pairs preanals	5 pairs preanals	3 pairs preanals, eye-shaped pores	reticulated, 6 pairs preanals, pair small circular pores	reticulated, 4 pairs preanals, crescentic pores
♂ No. of macrosetae on leg IV	one	--	--	--	three	one

All these species have 17 pairs of setae, nine of which are in the lateral rows. Setae Z₄ and Z₅ are the longest, and serrated to some degree. In addition, seta J₂ is present. There is at least one macroseta on the basitarsus of leg IV. The dorsal and ventrianal shields are reticulated to some degree. There is a pair of pores present on the ventrianal shield and three pairs of preanal setae. It should be noted that descriptions have often been made from a single specimen making definitive comparisons difficult.

2.3 Drawings of *N. californicus*

2.3.1 Materials and Methods

Mites taken from cultures of *N. californicus* were mounted on microscope slides in polyvinyl alcohol mountant (Appendix I), cleared on a hotplate (49°C for 2h) and examined under a compound microscope. Drawings were made, of the best specimens, using a camera lucida. For males and females, drawings were made of the dorsal and ventral shields, spermathecae, leg IV and chelicerae. The dorsal and ventral shields of the immature stages (larva, protonymph and deutonymph) were also drawn.

2.3.2 Results

The characteristics drawn were visible in all the specimens inspected, with the exception of a few individuals which were mounted without their chelicerae extended and for these the dentition could not be determined. Setae Z4 (M₂) and Z5 (L₉) on the dorsal shield are serrated. Chelicerae have 3 teeth on the moveable digit and 5 on the fixed digit, 2 of which are posterior to the pilus dentilis and 3 anterior. The fourth leg has one macroseta on the basitarsal segment. The female (Figure 2.2) has cup-shaped spermathecae and 3 sternal shields (sternal, genital and ventrianal). The sternal shield is entire, bearing 3 pairs of setae. The ventrianal shield has 3 pairs of pre-anal setae and a pair of distinct pores. The male (Figure 2.3) has an intricate spermodactyl on the moveable digit of the chelicera and 2 sternal shields (sternogenital and ventrianal). The ventrianal shield also bears 3 pre-anal setae and a pair of distinct pores.

The larval stage (Figure 2.4) is weakly sclerotised. There are fewest setae in the larval stage; setae are added with each moult and the fullest complement reached in the moult to adult. As the mite develops through the protonymph and deutonymph stages (Figures 2.5 and 2.6), there is greater sclerotisation of the ventral surface. In addition the dorsal surface area increases, and the distance between the setae becomes greater. In all the immature stages distinctive eye-shaped pores were visible on the ventrianal shield.

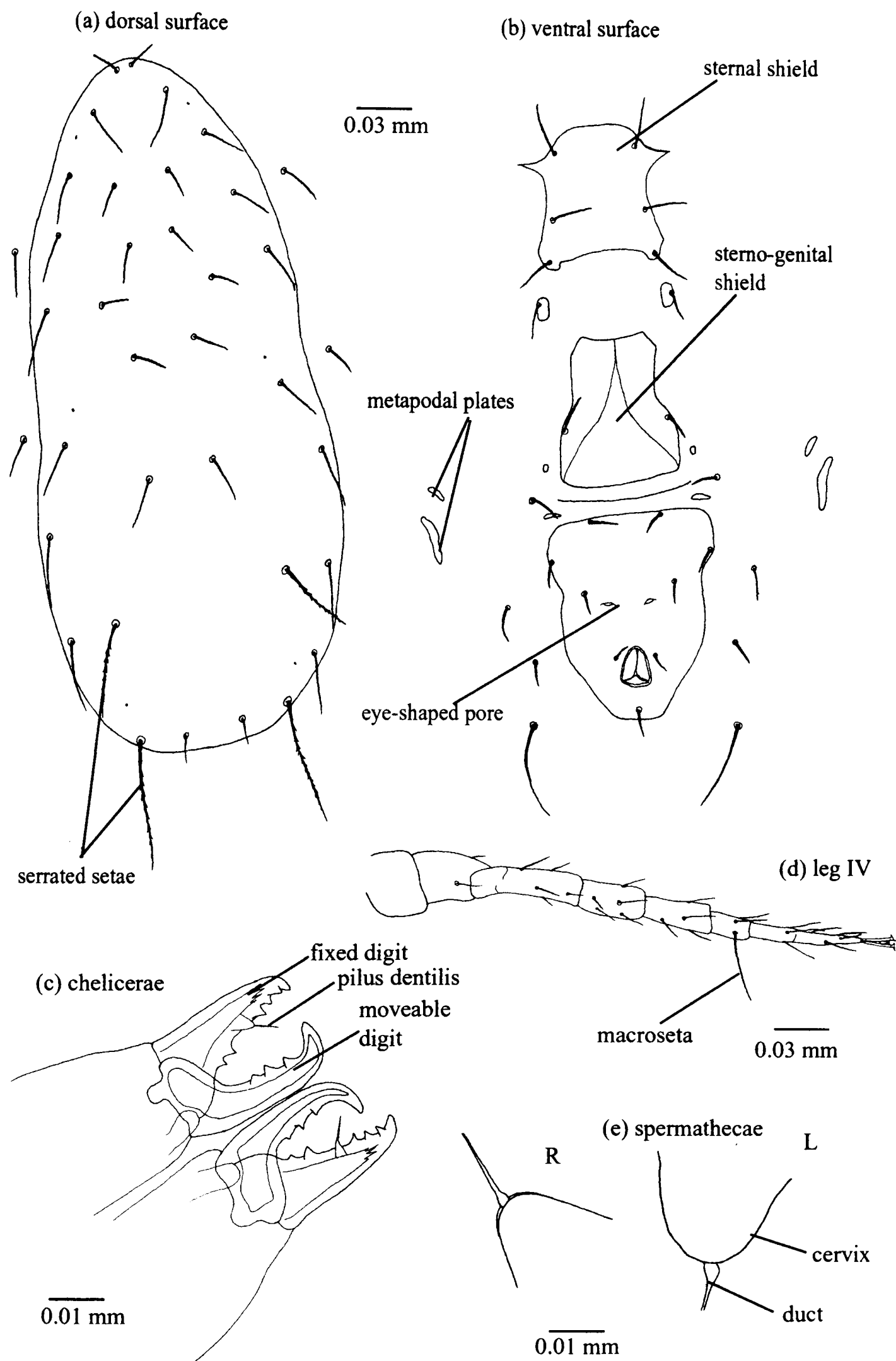


Figure 2.2 Adult female *N. californicus*: (a) dorsal and (b) ventral surfaces, (c) chelicerae, (d) leg IV and (e) spermathecae

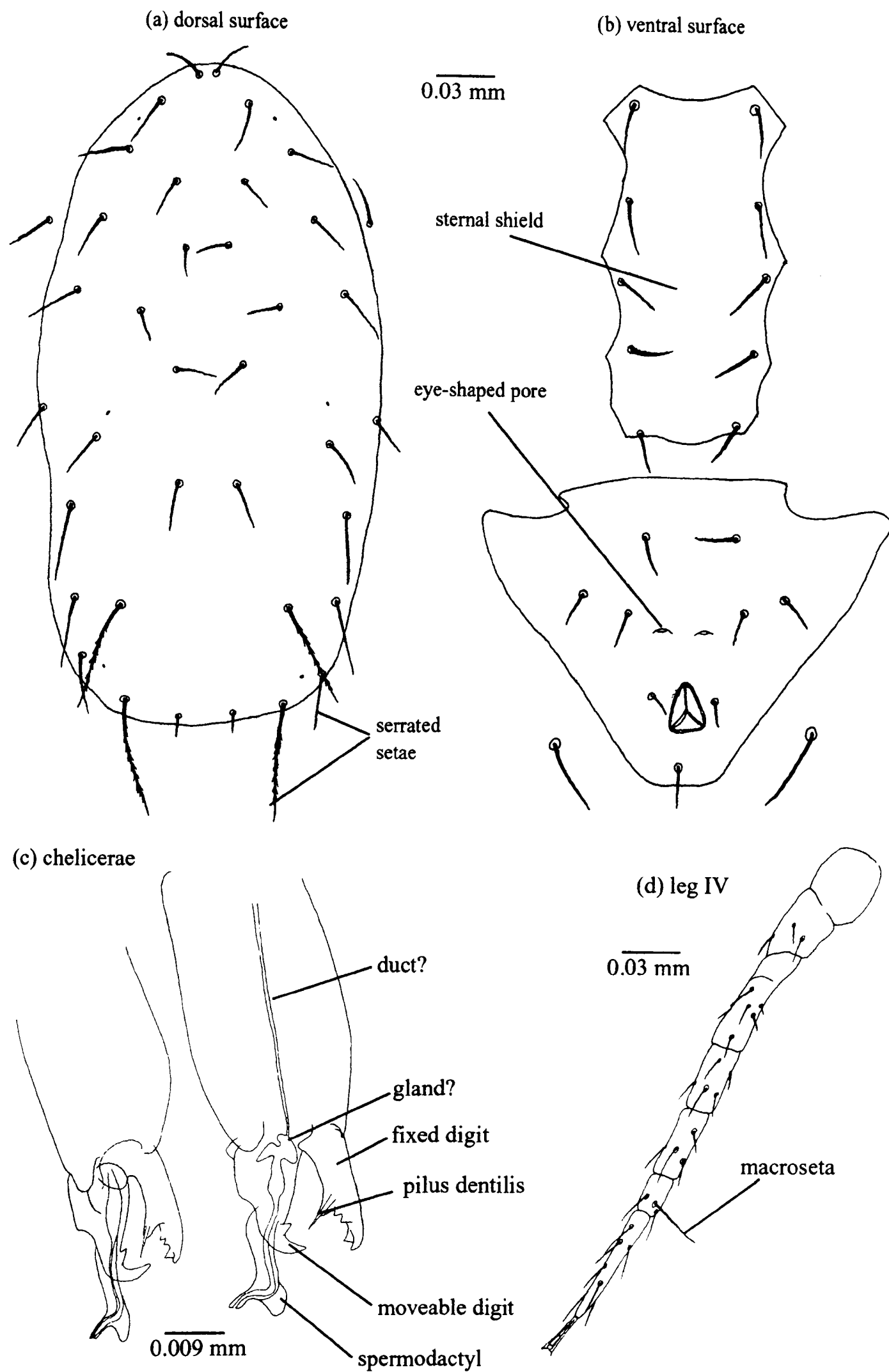


Figure 2.3 Male *N. californicus*: (a) dorsal and (b) ventral surfaces, (c) chelicerae and (d) leg IV

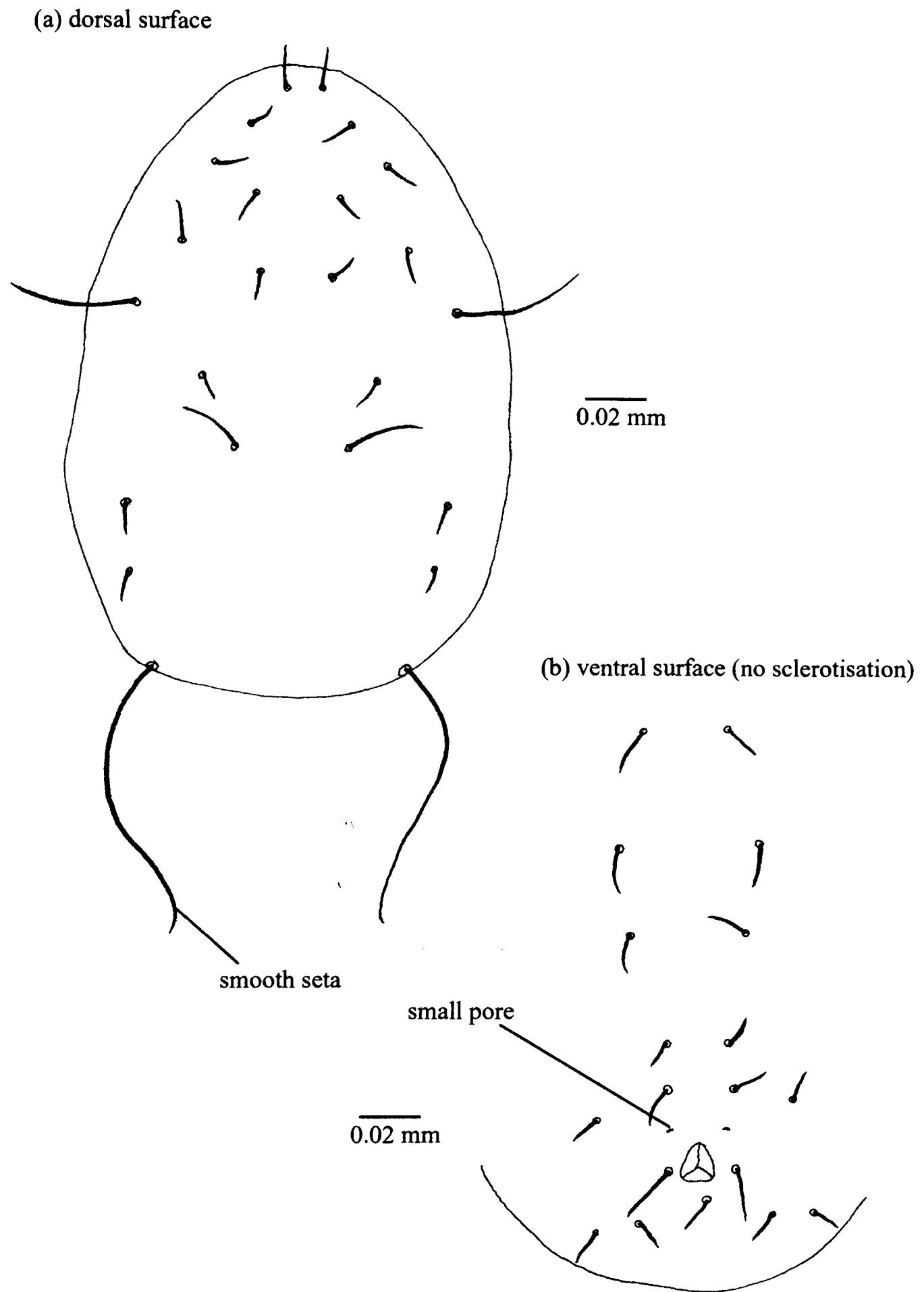


Figure 2.4 Larva of *N. californicus*: (a) dorsal and (b) ventral surfaces

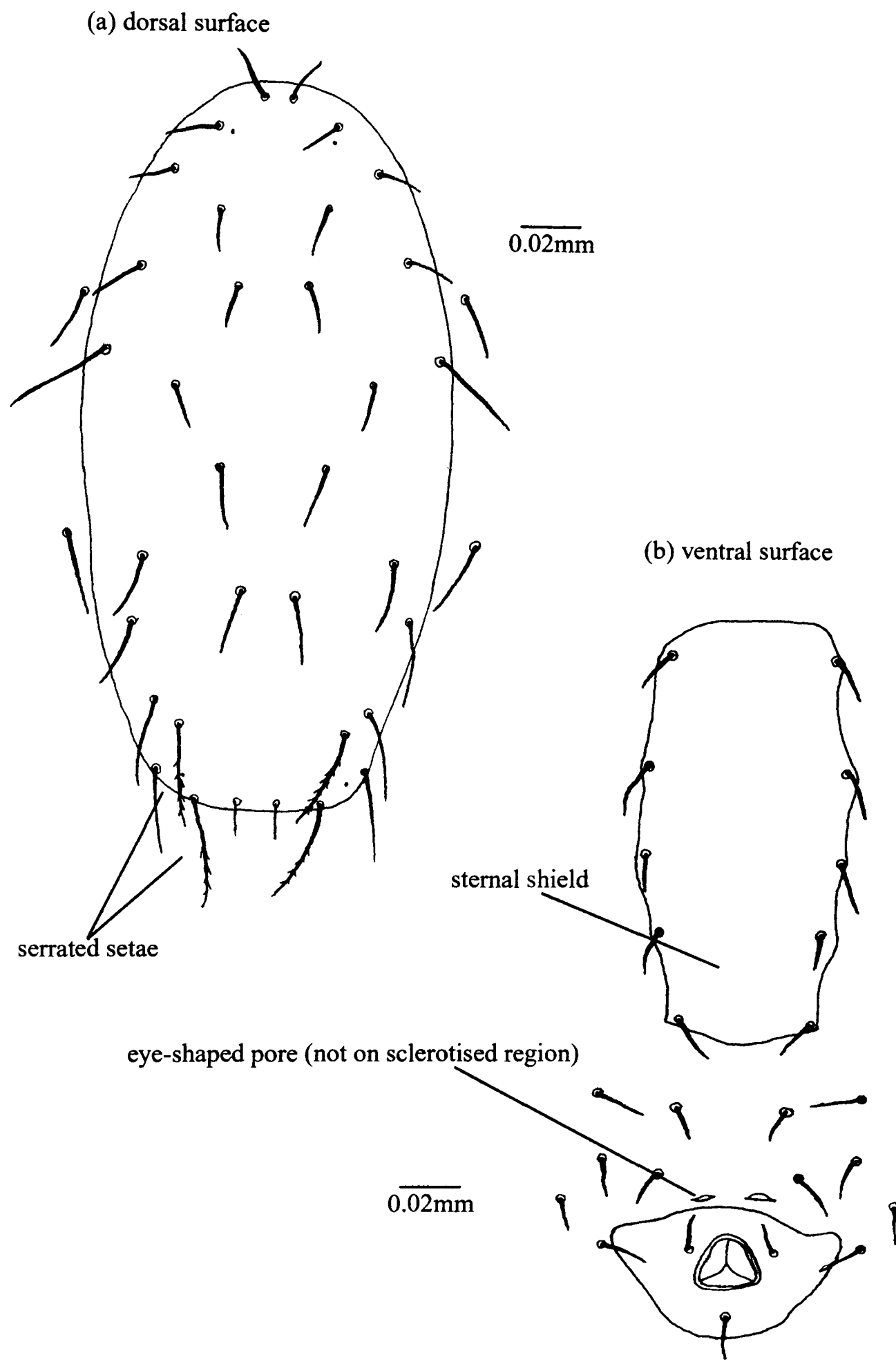


Figure 2.5 Protonymph of *N. californicus*: (a) dorsal and (b) ventral surfaces

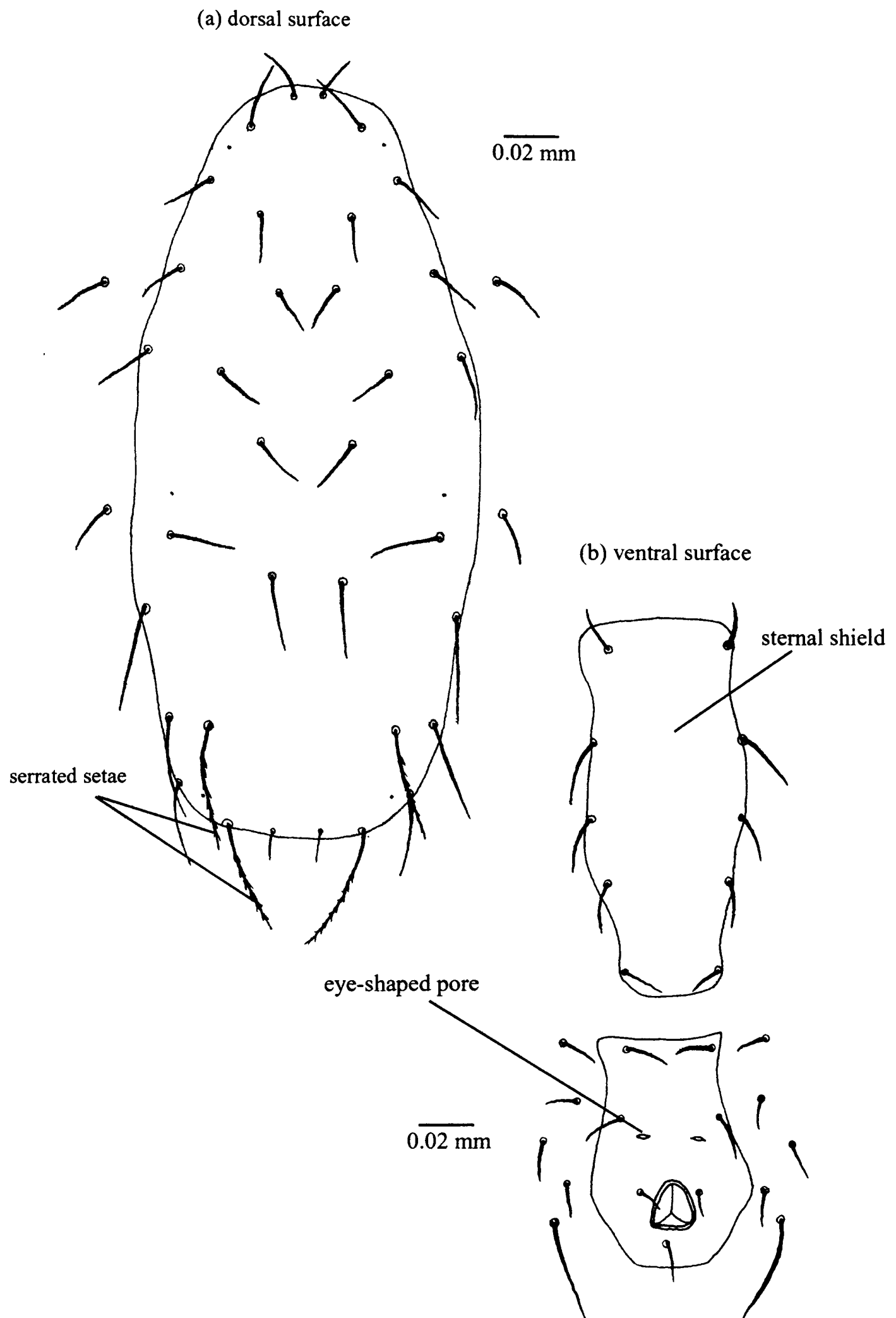


Figure 2.6 Deutonymph of *N. californicus*: (a) dorsal and (b) ventral surfaces

2.4 The effect of preparation on spermatheca shape

2.4.1 Materials and Methods

The method used was adapted from Saito *et al.* (1999). Two species of phytoseiid mites were used, *N. californicus* and *Typhlodromus pyri* Scheuten. Thirty female *N. californicus* and 30 female *T. pyri* were taken from laboratory cultures and individually mounted in Hoyer's medium with two cotton threads, about 140 µm in thickness, placed either side of the mite. The depth of a phytoseiid mite, measured on a SEM photograph from the dorsal to the ventral surface at the thickest point, was approximately 135 µm (personal observation). Thus, the threads allowed the mite to be mounted in such a way that it was not flattened by the coverslip. The slides were heated on a hot plate (49°C for 2h) to clear the mites and they were then examined under a compound microscope. The length and width of the dorsal shield (measured at seta S₂ (L₆)) and the cervix of the spermatheca were measured using an eyepiece graticule. The shape of the spermathecae were drawn using a camera lucida attached to the compound microscope. The coverslips were then removed with steam and the mites remounted in Hoyer's medium on new microscope slides. Twenty mites of each species were remounted without the threads and a 10g weight placed on each coverslip to flatten the mites. Preliminary experiments determined that this weight was sufficient to flatten the mite, without damaging the dorsal shield. Ten mites of each species were remounted as before, with threads, to assess the possibility that distortion of the spermatheca occurred due to handling. Once remounted the mites were again heated on the hot plate for 2h. The mites were re-examined, the length and width of the dorsal shield and the cervix of each spermatheca was again measured and the shape of the spermathecae drawn.

2.4.2 Results

More details were visible in the shape of the flattened spermathecae than those mounted with threads, and in many cases the major duct, vesicle and spermatophores were visible (Figure 2.7). The shape of the spermathecae in the flattened preparations was also more consistent and distinctive. When comparing the spermathecae of *N. californicus* and *T. pyri* from the original preparations (mounted with threads) there was little difference in shape, whereas in the flattened preparations distinctive differences were seen between the two species.

Table 2.3 shows the mean measurements of spermatheca cervix, dorsal shield length and width before and after remounting, the mean difference and the percentage increase in size. The number of individuals varies slightly as a few were inadvertently mounted sideways, lost during the remounting process, or did not have both spermathecae visible. The change in measurements (before and after remounting) were significant for all of the characteristics examined in both *N. californicus* and *T. pyri* that were remounted and flattened (paired t-test, $p < 0.05$). There was no significant difference in measurements for the mites that were remounted with threads under the coverslips, except for the dorsal shield width of *T. pyri* (paired t-test, $p < 0.05$).

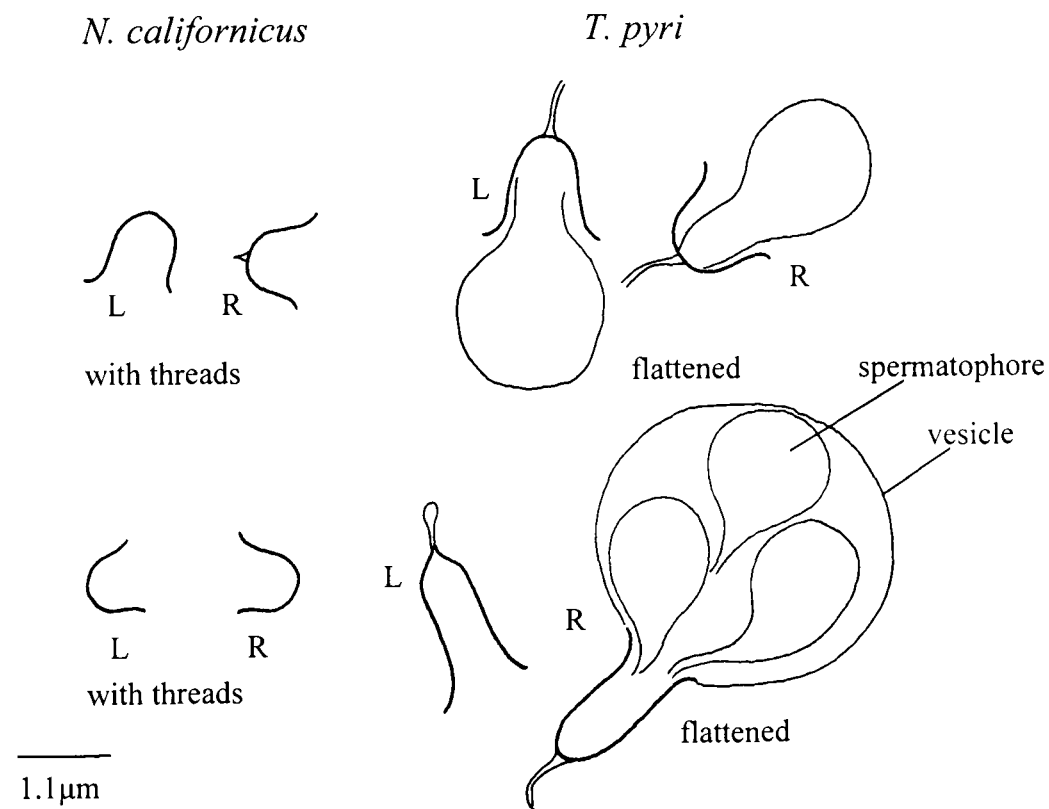


Figure 2.7 Drawings of the spermathecae from the same individual mite of two species of phytoseiid when mounted with threads under the coverslip and when flattened.

Table 2.3 Measurements of spermatheca cervix and dorsal shield length and width, mean difference and percentage increase.

		initial mounting		remounted		mean difference (μm)	% increase	sample size
		mean size (μm)	sed	mean size (μm)	sed			
<i>T. pyri</i> flattened	spth	7.5	0.9	10.8	1.2	3.2	42.7	25
	DSL	327.5	5.6	339.3	7.4	11.8	3.6	14
	DSW	180.8	5.2	187.4	5.3	6.6	3.7	14
<i>T. pyri</i> remounted with threads	spth	7.6	1.2	8.0	1.2	0.4	5.3	17
	DSL	329.8	7.6	329.3	9.2	-0.5	-	8
	DSW	180.8	5.2	184.6	5.8	3.8	2.1	8
<i>N. californicus</i> flattened	spth	9.2	1.9	13.7	2.6	4.5	48.9	30
	DSL	353.8	6.3	361.8	5.1	7.9	2.2	15
	DSW	184.9	8.5	194.4	5.0	9.5	5.1	15
<i>N. californicus</i> remounted with threads	spth	9.8	1.8	10.1	1.5	0.3	3.1	18
	DSL	352.1	8.4	353.4	9.5	1.3	0.4	9
	DSW	181.2	6.8	180.3	8.5	-0.9	-	9

spth = spermatheca cervix (viewed dorsally) NB sample size is greater as there are two spermathecae per individual. In some individuals only one spermatheca was clearly visible.
DSL = dorsal shield length
DSW = dorsal shield width

2.5 Measurements of morphological characteristics

2.5.1 Materials and Methods

Mites were mounted on microscope slides in polyvinyl alcohol mountant and cleared on a hotplate (49°C for 2h). The mites were examined with a compound microscope and measurements were taken using an eyepiece graticule. The morphological features measured were: length and width of dorsal and ventrianal shields, length of the macroseta on leg IV, length of setae J2 (D₅) and Z5 (L₉) and the spermatheca cervix in females. These setae were selected because Sabelis and Bakker (1992) proposed that the dorsal chaetotaxy of phytoseiid mites may contribute to their ability to manoeuvre through tetranychid webbing. They suggested two categories of setae for protection: margino-dorsal and mid-dorsal. These setae represent one from each of these categories; J2 is a mid-dorsal seta and Z5 a margino-dorsal seta. Measurements were taken from 89 female and 23 male *N. californicus*. A few immature stages were also measured.

2.5.2 Results

The mean measurements, with 95% confidence limits, are shown in Table 2.4. Where no confidence limits are shown, only one measurement was taken. Immature phytoseiids are challenging to mount and weakly sclerotised, making measurements difficult. Larvae do not have a fully sclerotised ventrianal shield (see Figure 2.4) and no fourth leg. Female mites are larger than males and the measurements reflect this, with the exception of ventrianal shield width. With the exception of the protonymph dorsal shield width and length, the confidence limits are reasonably low, therefore most of the population will display the same characteristics of size as these mites.

2.6 SEM study of chelicerae

Although the cheliceral dentition of *N. californicus* was noted by Flechtmann and McMurtry (1992) and was visible in some slide preparations mentioned previously in this chapter, an SEM study was carried out to provide confirmation of cheliceral dentition and to examine some other morphological characteristics of *N. californicus*.

2.6.1 Materials and Methods

The method used to prepare the mites for the SEM was adapted from that of Flechtmann and McMurtry (1992). Ten females of *N. californicus* and ten of *T. pyri* were transferred from cultures to a cavity slide containing water. The slide was heated on a hotplate, set at 49°C, to kill the mites and ensure that the chelicerae were extended. The mites were transferred to glass vials and fixed in 3% gluteraldehyde in 0.05M phosphate buffer for 24h. They were then transferred to plastic specimen processing pots (Agar Scientific Ltd.); these are perforated and allow solutions to wash through them without losing the sample inside. The processing pots were only suitable for use with female mites, as smaller stages fell through the perforations. The processing pots fit inside a 7ml glass vial. Thus, old fluid could be removed from outside the processing pot and fresh fluid added into it. After fixing and placing in the processing pots, the mites were dehydrated through a series of alcohol and acetone rinses (Appendix I). The specimens were dried in a critical point dryer (Polaron E3000) for 1h. They were then gold sputter-coated (Nanotech Sempreg 2) and examined in a scanning electron microscope (Hitachi S430). Photographs were taken on a 35mm camera.

Table 2.4 Mean measurements of a selection of morphological characteristics of *N. californicus*

stage	mean measurement in $\mu\text{m} \pm 95\%$ confidence limits							n
	dorsal shield length	dorsal shield width	ventrianal shield length	ventrianal shield width	leg IV macroseta	seta J2 (D ₅)	seta Z5 (L ₉)	
female	365.94 \pm 2.85	182.06 \pm 2.30	123.08 \pm 1.48	105.80 \pm 1.60	49.30 \pm 1.72	27.82 \pm 1.21	66.74 \pm 0.93	89
male	281.25 \pm 3.64	141.36 \pm 3.91	111.03 \pm 1.29	151.79 \pm 2.88	39.95 \pm 1.09	23.52 \pm 0.71	49.43 \pm 1.58	23
deutonymph	280.00 \pm 16.07	110.63 \pm 3.00	37.50	63.75	45.00 \pm 4.24	25.00 \pm 2.45	46.25 \pm 2.45	3
protonymph	222.50 \pm 29.80	126.25 \pm 32.96	41.25	63.75	40.00 \pm 2.45	22.50	35.00 \pm 2.45	3
larva	196.88 \pm 3.67	103.13 \pm 3.67	---	---	---	26.25	84.38 \pm 3.67	2

2.6.2 Results

The scale bars on the SEM photographs are in μm . Figures 2.8-2.10 show the side, front and ventral views respectively of the anterior region of *N. californicus* and the position of the chelicerae can be seen. Figures 2.11 and 2.12 show the chelicerae. The dentition was deduced from 5 individuals, and the best photographs are shown here. The dentition was 3 teeth on the moveable digit and 5 on the fixed digit (2 posterior to the pilus dentilis and 3 anterior). This was the same for all the individuals examined. Figure 2.13 shows the chelicerae of *Typhlodromus pyri*, which had different dentition to that of *N. californicus*: 2 teeth on the moveable digit and 3 on the fixed digit.

Figures 2.14 and 2.15 show a side and dorsal view of *N. californicus*. The setal distribution and shape of the dorsal shield can be seen. Figure 2.16 shows the macroseta on leg IV, Figure 2.17 the ventrianal shield, and a magnified view of the distinctive pores are shown in Figures 2.18 and 2.19. It is interesting to note that the pores seemed to be openings into the ventral surface of the mite and not merely folds in the cuticle.

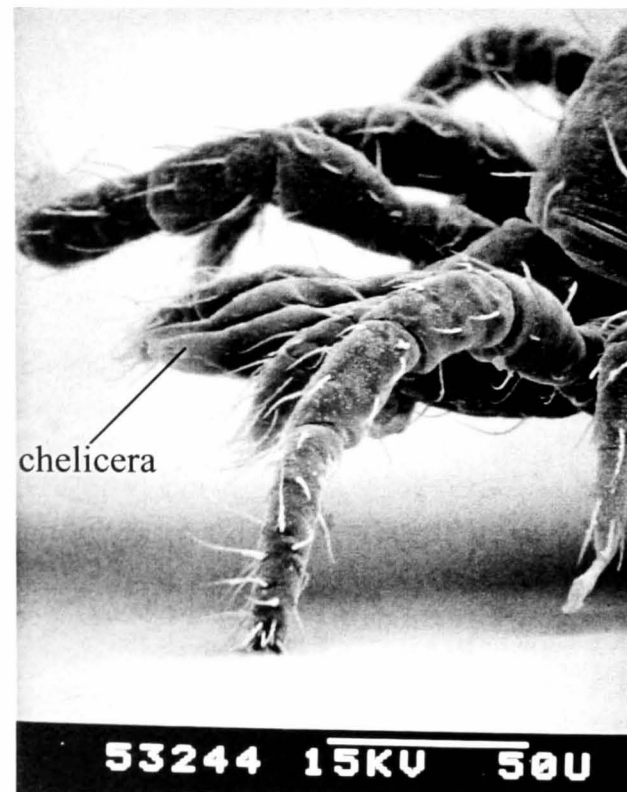


Figure 2.8 *Neoseiulus californicus* with chelicerae extended (viewed from the side).

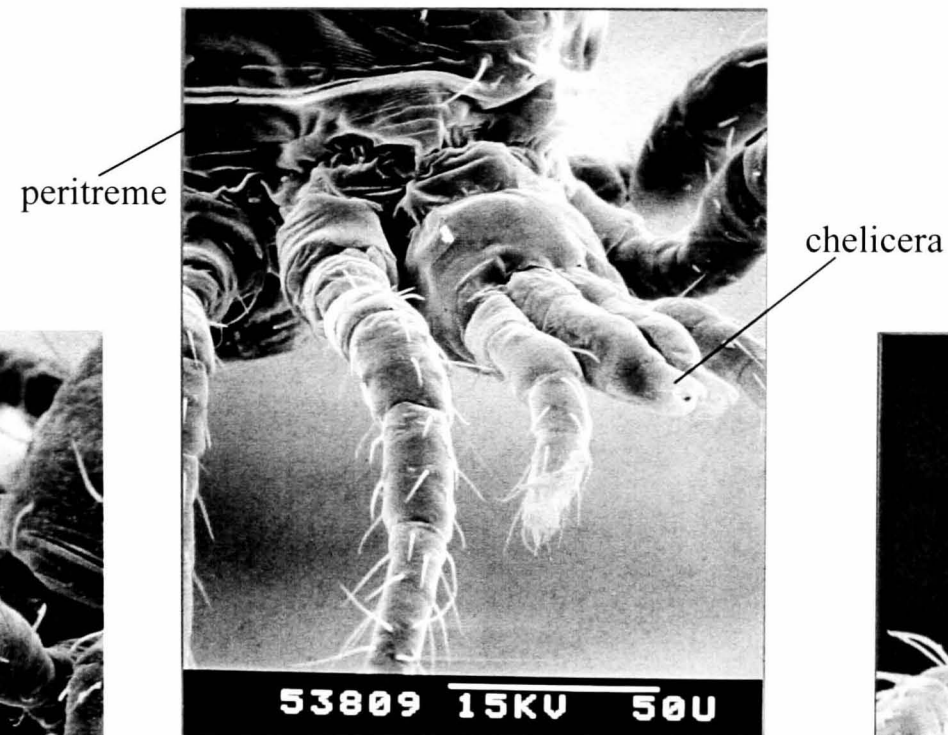


Figure 2.9 *Neoseiulus californicus* with chelicerae extended (viewed from the front).

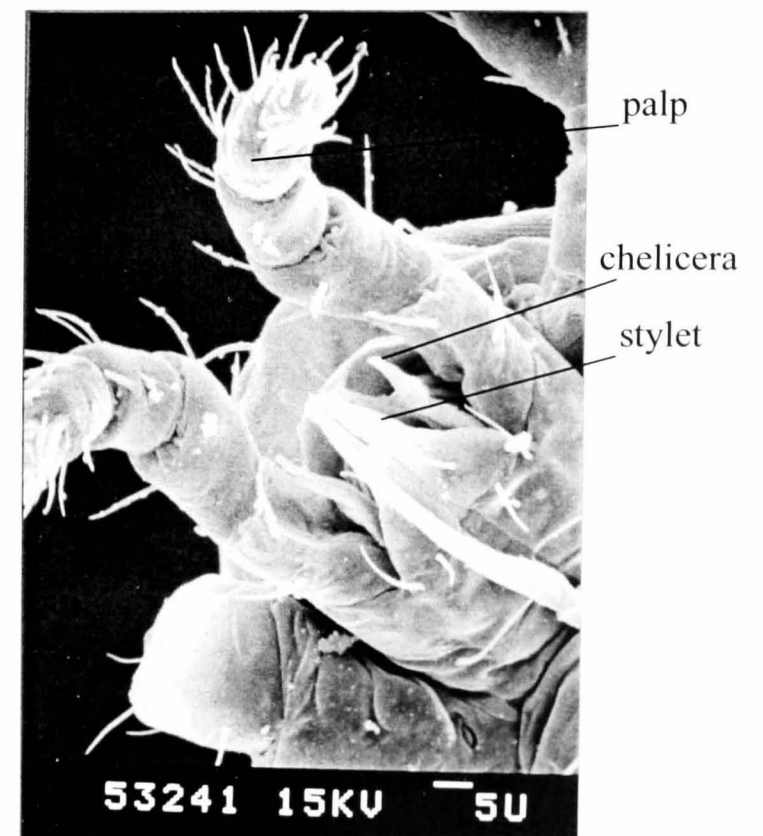


Figure 2.10 *Neoseiulus californicus* gnathosoma: ventral surface.



Figure 2.11 *Neoseiulus californicus* chelicera:
3 teeth on moveable digit (2 clearly visible), 5 teeth on fixed digit, 2 posterior to pilus dentilis and 3 anterior.



Figure 2.12 *Neoseiulus californicus* chelicera.

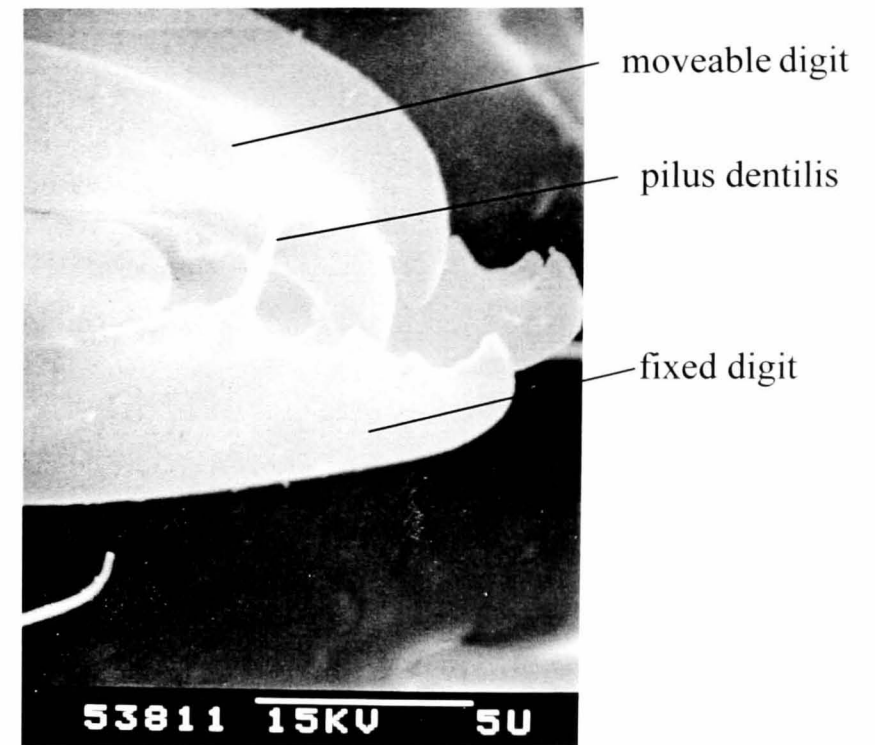


Figure 2.13 *Typhlodromus pyri* chelicerae:
2 teeth on moveable digit (one is hidden behind the pilus dentilis), 3 on fixed digit anterior to pilus dentilis, none posterior.

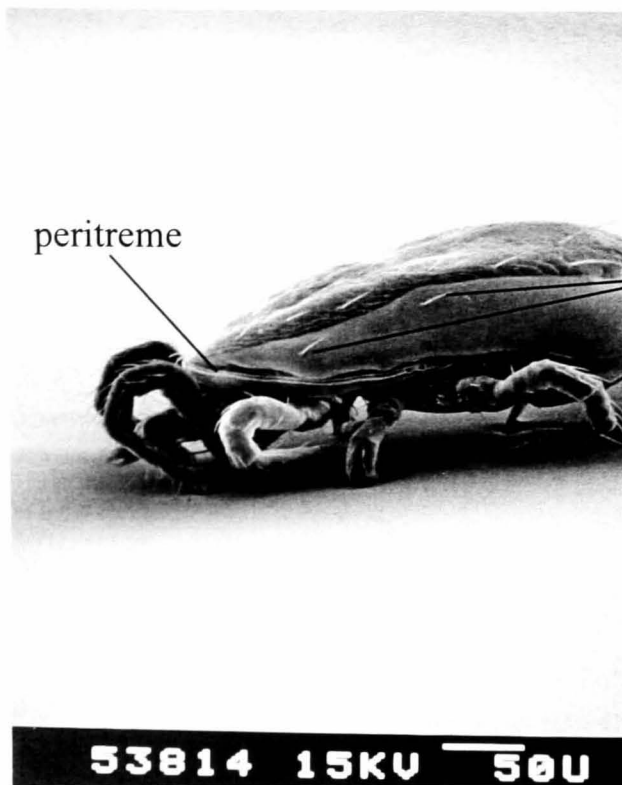
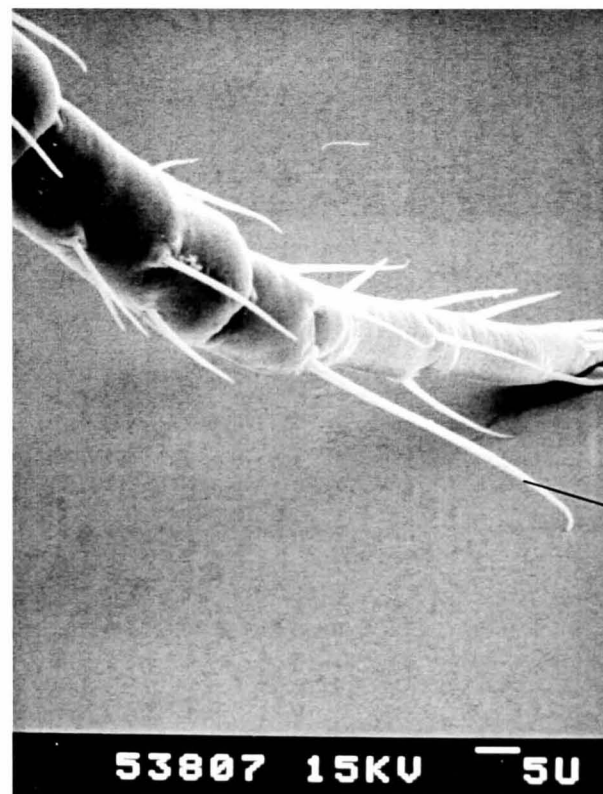


Figure 2.14 *Neoseiulus californicus* side view.

setae on lateral integument
(r3 (S_1) & R1 (S_2))

serrated dorsal setae
(Z4 (M_2) & (Z5 (L_9)))

macroseta



macroseta

Figure 2.16 Leg IV of *N. californicus*.

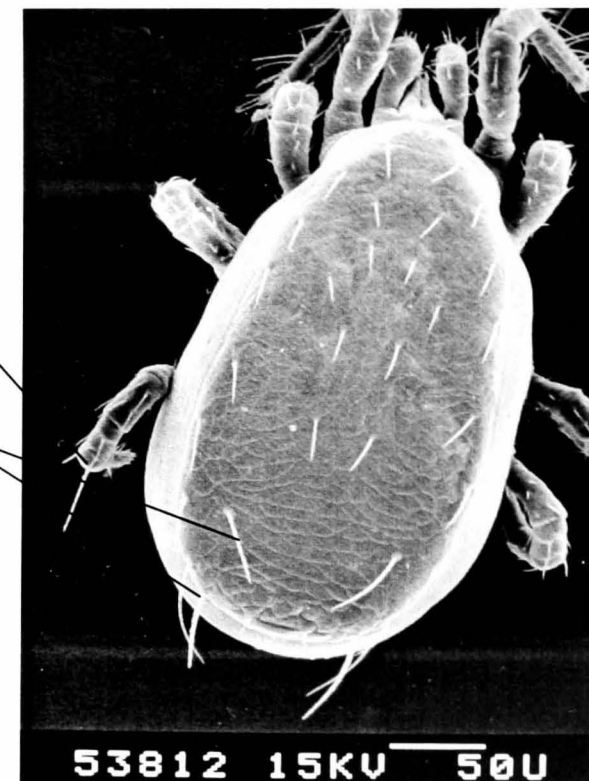


Figure 2.15 Dorsal view of *N. californicus*. Macroseta of leg IV on left side is visible.

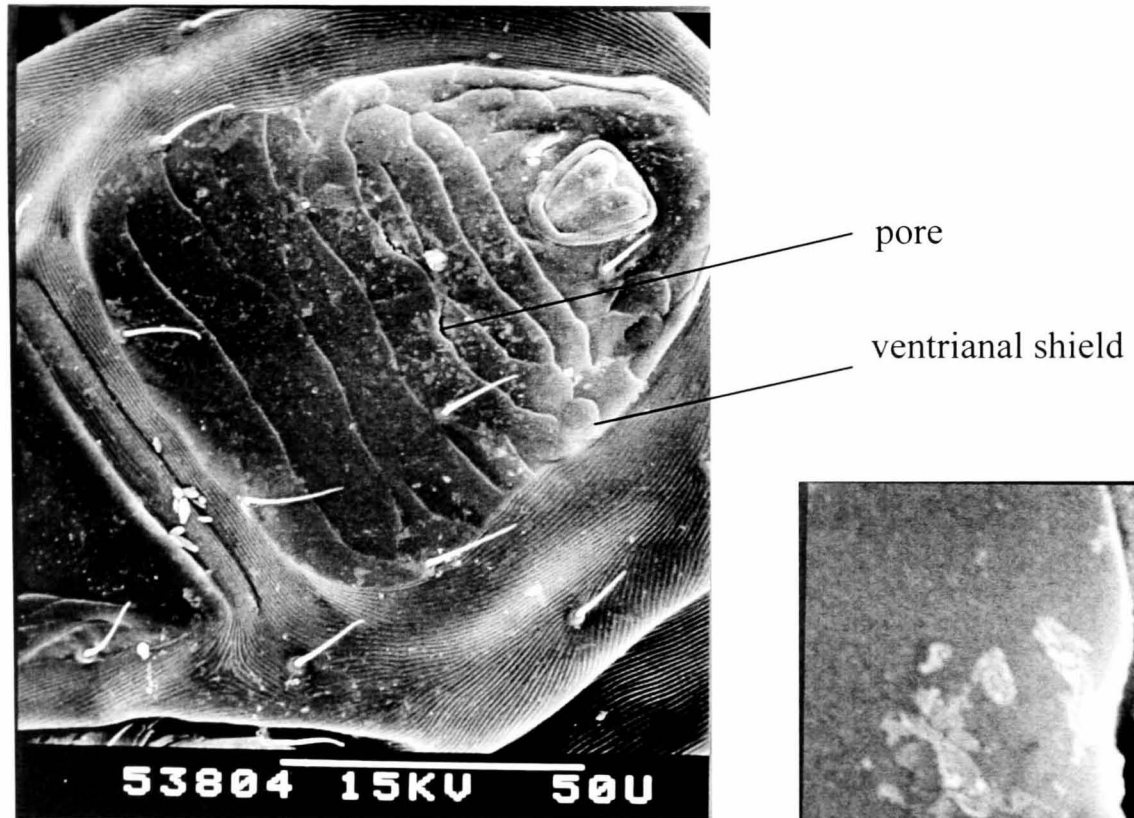
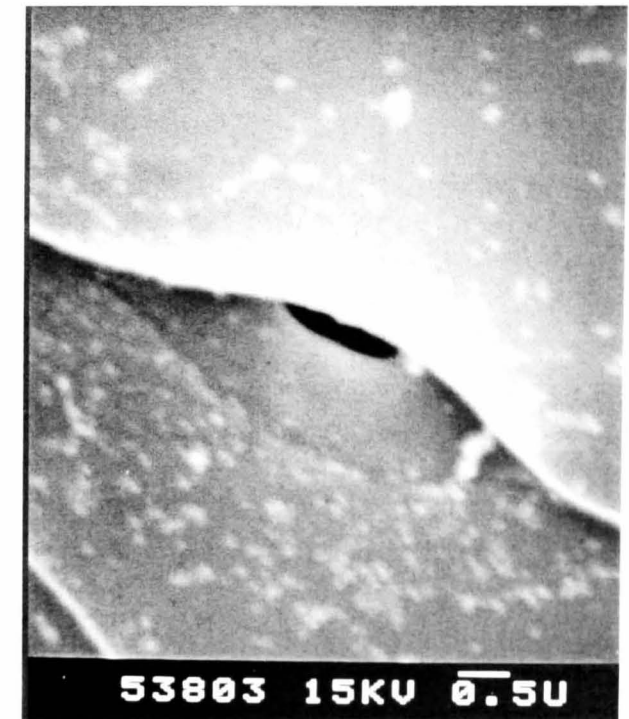
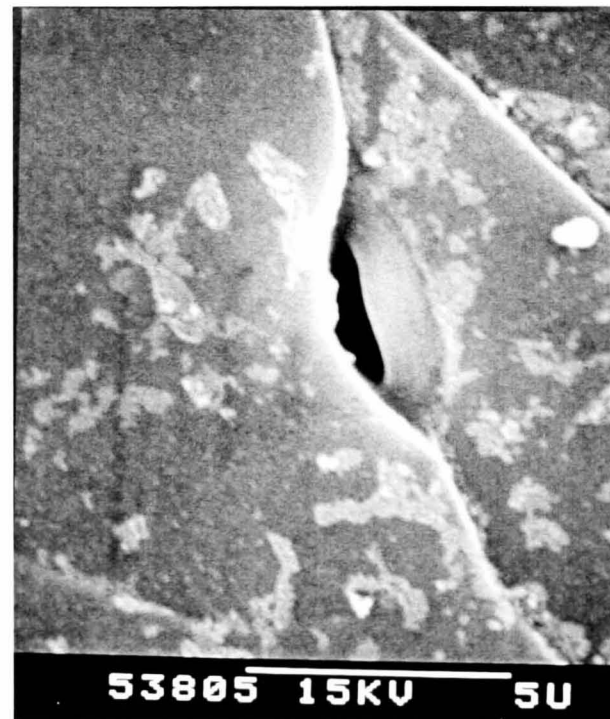


Figure 2.17 Ventrianal shield of *N. californicus*.



Figures 2.18 & 2.19 Pores on the ventrianal shield of *N. californicus*.

2.7 Discussion

On examination of the literature, and supported by the findings of this chapter, it is reasonable to conclude that *N. californicus* is synonymous with *T. mungeri*, *A. californicus* and *N. chilensis*. However, synonymy with either *T. marinus* or *N. fallacis* cannot be supported. The male preanal setal homology was the same for all the *N. californicus* studied over three years (>350 individuals). This lends weight to the argument that *T. marinus* is not synonymous with *N. californicus*; Chant's (1959) description is of 5 preanal setae in the male. Schuster and Pritchard (1963) describe male *N. californicus* as having 3 or 4 preanal setae, this is incorrect and Kreiter and de la Bourdonnaye's (1993) description of 3 preanals in the male should be accepted.

The cheliceral dentition of *N. californicus* was established by examination under the light microscope and the SEM. The cheliceral dentition agrees with that noted by Flechtmann and McMurtry (1992). Many structures were easily examined and clearly visible under the SEM. The SEM also presented an incontrovertible method of examining chelicerae. However, it was fairly difficult to ensure that the chelicerae were extended and in a position to be examined. It is also time consuming and expensive; therefore, this technique would possibly be best utilised for new species or where the dentition is unconfirmed.

The SEM showed that the pores in the ventrianal shield were openings into the ventral surface of the mite. However, their function is not known. It raises the question as to why some species have pores in the ventrianal shield and others do not. Of the species which have pores in the ventrianal shield, these can vary from very small and circular to large and crescent-shaped (as they are in *N. californicus*). Many species have pores on their dorsal shield, often small and spherical. Are they structures pertaining to gaseous exchange? Or perhaps pheromone secretion? All mites of the Astigmata family have a pair of opisthonotal glands which secrete volatile chemicals (Sakata *et al.*, 1997), and these have been shown to function as alarm, aggregation or sex pheromones. It is highly likely that phytoseiid mites also have pores in their surface integument to allow chemical secretion. Perhaps a future study may involve sectioning dorso-ventrally the area of the ventrianal shield that the pores occur in and TEM examination. It is interesting to note that the distinctive eye-shaped pores are present in all the immature stages of *N. californicus*. Whilst there are other phytoseiids which also have

eye-shaped pores (e.g. *Amblyseius reductus* Miedema, *N. fallacis*, *Neoseiulus collegae* (DeLeon)), it may be possible to use this as an identifying feature especially when in conjunction with some background knowledge about typical adult phytoseiid fauna taken from a particular locality.

The investigation into the effect of preparation on spermatheca shape could provide an explanation for differences observed in size and shape of spermathecae in phytoseiids. For a species such as *N. californicus*, whose spermathecae are a relatively straightforward cup-shape, the mounting technique can make a significant difference to the shape and size of the spermatheca. The spermatheca cervix size (9.2 μm) of *N. californicus* when mounted initially, increased by 4.5 μm (an increase of 49%) when the mite was remounted and flattened. The cervix size recorded by Schuster and Pritchard (1963) of 11 μm is intermediate. *Typhlodromus pyri* has a longer, thinner shaped spermatheca which may be expected to be less affected by mounting technique. However, the degree of flattening was an important consideration for this species too, as the difference measured was approximately 3 μm ; i.e. a percentage increase of 43%. The size recorded for this species by Dosse (1958b) was 10 μm . Thus, it is important to consider the details of mounting technique when consulting the literature. In his description of *Amblyseius potentillae*, based on a survey of the literature, Miedema (1987) notes that the appearance of the spermatheca is variable in different mounted specimens. The increase in detail that can be observed once a specimen is flattened is an important consideration when preparing mites for high-powered microscopic examination. If the mite is prepared such that the spermatheca is flattened out, then the spermatheca assumes a characteristic shape that will differ from that of other phytoseiids.

It is interesting to note that characters such as the dorsal shield length and width may also change significantly with different mounting techniques. The dorsal shield is only slightly convex, but nevertheless it was shown that flattening will have an effect on its dimensions. The differences recorded for the dorsal shield length and width respectively were approximately 8 μm and 9.5 μm for *N. californicus* and 12 μm and 7 μm for *T. pyri*. Thus, given the smaller percentage increase in size (between 2 and 5%), differences for these characters may be less important in taxonomic studies in comparison to those for the spermatheca. In the present study the mean measurements for the dorsal shield length and

width of flattened *N. californicus* were approximately 362 μm and 194 μm respectively, and for *T. pyri* were 339 μm and 187 μm . Schuster and Pritchard (1963) note that the length and width of the dorsal shield of *N. californicus* was 355 μm and 170 μm respectively, and that of *T. pyri* was 325 μm and 180 μm .

Whilst these findings have implications for taxonomic purposes, it should be noted that the identification of phytoseiid mites relies on a combination of characters and a variation in dorsal shield length and width may not have a great impact. However, for spermathecae, the changes in shape resulting from mounting technique may lead to confusion in determining the identification of species.

In general, the measurements of morphological characteristics increase in size from larva to adult as do their sclerotised structures. The macroseta on leg IV and setae J2 and Z5 also get longer with each moult, with the exception of larvae. For these, J2 and Z5 are relatively longer, with Z5 being the longest in this stage. Sabelis and Bakker (1992) propose that dorsal chaetotaxy of phytoseiid mites may contribute to their ability to manoeuvre through tetranychid webbing. They suggest two categories of setae for protection: margino-dorsal (including Z5) and mid-dorsal (including J2 and Z5). It is possible that, owing to their smaller size and fewer setae, larvae are more vulnerable to entanglement in the strands of tetranychid webbing and that the evolution of comparatively longer setae in this stage is a response to the challenge of manoeuvring through prey webbing.

The measurements of the adult female *N. californicus* taken in this study are comparable with those published previously. Table 2.5 enables a quick comparison. The results for the dorsal and ventrianal shield dimensions lie between Gonzalez and Schuster's (1962) and Çobanoğlu's (1993) observations.

The 95% confidence limits for the present study (Table 2.4) show that there is little variation about the mean, except for the dorsal shield measurements for protonymphs. This is possibly due to the small sample size, and it is possible that one of the individuals was unusually small. An alternative possibility is that protonymphs show differentiation in size in relation to the sex of the adult. It would be necessary to examine larger numbers of protonymphs and be

able to separate the data into two distinct size classes to prove this hypothesis, as the measurements cannot be made on live individuals.

There are no drawings or measurements of the immature stages of *N. californicus* in the literature, thus those presented here represent a comprehensive depiction for this species. Further work could concentrate on elucidating the function of some regions of the mite, for example an SEM study on the nature of sensory structures of features such as the palps.

Table 2.5 Previously published measurements in μm (rounded to the nearest μm) for some morphological characteristics of adult female *N. californicus* and the results of the present study.

	dorsal shield		ventrianal shield		setae		
	length	width	length	width	leg IV macroseta	J2	Z5
Schuster & Pritchard (1963)	355	170	115	98	48	30	76
Gonzalez & Schuster (1962)	360	190	?	78	55	30	69
Çobanoğlu (1993)	382	225	132	107	30	---	59
present study	366	182	123	106	49	28	67

CHAPTER 3: OCCURRENCE OF *N. CALIFORNICUS* WORLDWIDE AND IN THE UK

3.1 The worldwide occurrence of *N. californicus*: a literature review.

3.1.1 Early records

Neoseiulus californicus was first described from citrus, in California in 1954 (McGregor, 1954), and Chant (1959) described *Typhlodromus californicus* and *Typhlodromus mungeri* from lemon fruit in California in 1953. Subsequently it was found in 1956 in Algeria and France on cultivated strawberries, leaf litter and a range of plants (Athias-Henriot, 1959). *Amblyseius chilensis* was described from S. America in 1958 and is 'morphologically indistinguishable' from *N. californicus* (Athias-Henriot, 1977). In 1965 *N. californicus* was discovered on strawberry plants in biological research plots in Orange County, S. California (McMurtry *et al.*, 1971; Oatman, 1971).

3.1.2 Europe

Individuals described as *N. californicus* have been discovered in laboratory collections labelled as 'Montpellier (Lavalette) on maize 25/08/1965' and 'St Marcel lès Valence (Drôme) on apple 23/08/1966' (Fauvel *et al.*, 1993). *Neoseiulus californicus* was found in a neglected orchard in Avignon, France in 1976 (Athias-Henriot, 1978). In 1971, it was present on citrus in the Mediterranean region (McMurtry, 1977).

In 1992, *N. californicus* was found to be one of the most abundant species of mite on apple, pear and peach in Navarra, Spain (Iraola *et al.*, 1994). Garcia-Mari *et al.* (1987) noted high numbers in vineyards of Valencia, Spain, during 1983 and 1984. From 1985 to 1992, a study was carried out in French vineyards encompassing all the wine-growing regions of France. *Neoseiulus californicus* was present in nine of the 15 regions, and was also discovered on wild *Rubus* sp. (e.g. blackberry) near to the vineyards in Champagne (Kreiter *et al.*, 1993; Sentenac *et al.*, 1993). *Neoseiulus californicus* appeared 'spontaneously' in nearly all the main fruit growing regions in France, and occupies up to 80% of apple and pear (*Pyrus communis* L.) orchards in the south (Fauvel *et al.*, 1993). In north-east Portugal, during 1993, it was found in both sprayed and

unsprayed orchards (Espinha *et al.*, 1995) and was found to be one of the predominant predatory mite species on bean (*Phaseolus* sp.) all over Portugal in 1994 (Ferreira & Carmona, 1994).

In 1986, *N. californicus* was found on leaves and on the plants under vines in the Tuscany region of Italy (Ligouri & Castagnoli, 1989). It was collected from bean, melon (*Cucumis* sp.) and watermelon (*Citrullus* sp.) in Lazio, in 1991, where it was also found on some wild plants (*Amaranthus retroflexus* (pigweed/amaranth), *Solanum nigrum* (deadly nightshade), *Trifolium* sp. (clover)) (Calvitti & Tsolakis, 1992). A survey of phytoseiid mites in Italian vineyards, in 1994, found *N. californicus* to be prevalent (Nicòtina, 1996), and during a study of soybean (*Glycine max*) in Italy it was found to be the only predatory mite with a positive association with *Tetranychus urticae* (Castagnoli *et al.*, 1993).

In Switzerland, six species of predatory mites were found in two strawberry plots, and of these, *N. californicus* and *N. fallacis* may have been present due to artificial or accidental introduction (Baillod *et al.*, 1995). *Neoseiulus californicus* ‘spontaneously appeared’ on experimental plots of gerbera plants, in Poland in 1994, and was recorded as a new species for the Polish fauna (Kielkiewicz & Witul, 1995).

3.1.3 South America

Neoseiulus californicus was collected in Santa Rosa, Peru from peach (*Prunus persica*) and *Manihot utilissima* (cassava) in 1976 (El-Banhawy, 1979). It was recorded from *Prunus* sp. in Columbia from Bogota in 1986, and Valle del Cauca in 1984; previous records were made from Argentina, Chile, Guatemala and Peru, and as *A. chilensis* from Brazil and Uruguay (de Moraes & Mesa, 1988). Müther *et al.* (1996) found *N. californicus* in the Rio Negro province of Argentina during 1990 to 1995. It was present in the majority of commercial apple orchards in Uruguay, where it was predominantly predatory on *P. ulmi* (Bruhn & Beltrame, 1981).

In Valparaíso, Chile, *N. californicus* was found in 1961 on leaf litter and on vines (where it was feeding on *Brevipalpus chilensis*) (Gonzalez & Schuster, 1962). It was also collected from moss on the bank of irrigation channels in an orchard, river plants, *Rubus ulmifolius* (blackberry)

and peach in La Cruz, and an abandoned apple orchard in Talca (Gonzalez & Schuster, 1962). It is widely distributed in the central zone of the country, on plants of the Rosaceae family, grapevines and ornamentals (Gonzalez, 1971).

3.1.4 Africa & Asia

The first record in the literature of *Neoseiulus californicus* from Africa was on peach in the Canary Islands in 1985 (Pande *et al.*, 1989). Since 1965, *N. californicus* has been found in the Maghreb region of NW Africa and Southern Europe (Fauvel *et al.*, 1993). Ueckerman and Loots (1988) list it as an African phytoseiid species. An experimental release investigation in Africa in the 1980's involved the release of many phytoseiid species in field sites in the main cassava growing regions. Columbian *N. californicus* were released in Ghana, Kenya and Nigeria in 1987, and Bénin, Burundi, Gabon, Uganda and Zaire in 1988. The mites were found in Benin, Kenya and Nigeria the following year in less than half of the release sites (Yaninek *et al.*, 1993).

Neoseiulus californicus was recorded in the Chiba Prefecture, Matsudo, China in 1983, on *Coculus orbiculatus* and *Menispermum dauricum* (Amano, 1994). It was subsequently found on *Hydrangea* sp., and Kudzu-vine in 1984 and Creeping Lady's-sorrel (*Rumex* sp.), pear and vetch (*Vicia sativa*) in 1991 (Amano, 1994). It has also been recorded in Japan (Ehara, 1966).

Table 3.1 lists, in chronological order, records of *N. californicus* (with original generic name) with its locality and host plant. Figures 3.1-3.4 are world maps with the locality of these records shown.

Table 3.1: The chronological order of records of *N. californicus* (or mites with suggested synonymy, see Chapter 2, section 2.2). The country, host plant and citation are given.

Year	location/country	host plant	mentioned as:	author(s)
1953	Whittier, California, USA	<i>Citrus</i> sp.	<i>T. californicus</i> <i>T. mungeri</i>	McGregor, 1954
1954			<i>A. californicus</i>	Ueckerman & Loots, 1988
1956	Fort de-l'Eau, Algeria Maison-Carrée, Maison Blanche & Béni-Messons, France	<i>Cydnodon dactylon</i> cultivated strawberries, leaf litter, <i>Phaseolus coccineus</i> , <i>Plantago</i> <i>major</i> , <i>Paspalum distichum</i> , <i>Dactylis</i> <i>glomerata</i> and <i>Potentilla reptans</i>	<i>A. mungeri</i>	Athias-Henriot, 1959 "
1958	San Diego & Riverside counties, California, USA d'Antibes & Vaucluse, France	citrus pecan litter	<i>A. californicus</i>	Schuster & Pritchard, 1963
1958	Chile	<i>Eichhornia crassipes</i> (glasshouse)	<i>T. chilensis</i>	Dosse, 1958a
1958	Valgo, Chile	(not given)	<i>C. californicus</i>	Athias-Henriot, 1977
1961	Cuesta del Pucalán, Chile	leaf litter, vines, moss on river bank, aquatic plants, <i>Rubus ulmifolius</i> , peach, abandoned apple orchard	<i>A. chilensis</i>	Gonzalez & Schuster, 1962.
1965	Orange County, California, USA	strawberry	<i>A. californicus</i>	McMurtry <i>et al.</i> , 1971 Oatman, 1971
1965	Montpellier, France	maize	<i>N. californicus</i>	Fauvel <i>et al.</i> , 1993
since	Maghreb region, NW Africa	(not given)	<i>N. californicus</i>	Fauvel <i>et al.</i> , 1993
1965	& S Europe			
1966	St Marcel lès Valence, France	apple	<i>N. californicus</i>	Fauvel <i>et al.</i> , 1993
1971	Sagunto & Alcira, Spain	citrus	<i>A. californicus</i>	McMurtry, 1977
1971-76	Uruguay	apple	<i>A. chilensis</i>	Croft <i>et al.</i> , 1976
1976	Santa Rosa, Peru	peach, <i>M. ultissima</i>	<i>A. californicus</i>	El-Banhawy, 1979
1976	Avignon, France	vegetation in neglected orchard	<i>C. californicus</i>	Athias-Henriot, 1978

1983-84	Chiba Prefecture, Matsudo, China	<i>Coculus orbiculatus</i> , <i>Menispermum dauricum</i> <i>Hydrangea</i> sp., kudzu-vine	<i>A. californicus</i>	Amano, 1994
1983-84	Valencia, Spain	vineyards	<i>A. californicus</i>	Garcia-Mari <i>et al.</i> , 1987
1984	Valle del Cauca, Columbia	<i>Prunus</i> sp.	<i>A. californicus</i>	de Moraes & Mesa, 1988
1985	Canary Islands	peach	<i>A. californicus</i>	Pande <i>et al.</i> , 1989
1985-92	Bourgogne, Champagne & Mediterranean region, France	vineyards, wild <i>Rubus</i> sp.	<i>N. californicus</i>	Kreiter <i>et al.</i> , 1993
1986	Metropolitan region, Chile	raspberry	<i>A. chilensis</i>	Guilleminot & Apablaza, 1986
1986	Tuscany, Italy	vines & weeds underneath	<i>A. californicus</i>	Ligouri & Castagnoli, 1989
1986	Bogota, Columbia	<i>Prunus</i> sp.	<i>A. californicus</i>	de Moraes & Mesa, 1988
1987	Ghana, Kenya & Nigeria	released on cassava	<i>A. californicus</i>	Yaninek <i>et al.</i> , 1993
1988	Bénin, Burundi, Gabon, Uganda & Zaire	released on cassava	<i>A. californicus</i>	Yaninek <i>et al.</i> , 1993
1990-92	General Roca, Rio Negro, Argentina	apple	<i>N. californicus</i>	H. Vogt – pers. comm.
1991	Chiba Prefecture, Matsudo, China	creeping lady's sorrel, pear, vetch	<i>A. californicus</i>	Amano, 1994
1991	Lazio, Italy	bean, melon, watermelon, <i>Amaranthus retroflexus</i> , <i>Solanum nigrum</i> , <i>Trifolium</i> sp.	<i>A. californicus</i>	Calvitti & Tsolakis, 1992
1991	Kent & Hampshire, England	strawberry	<i>A. californicus</i>	Solomon, unpub.
1992	Navarra, Spain	apple, pear, peach	<i>A. californicus</i>	Iraola <i>et al.</i> , 1994
1993	Southern France	80% of apple & pear orchards	<i>N. californicus</i>	Fauvel <i>et al.</i> , 1993
1993	Lamego, Tavora & Dourno, Portugal	apple	<i>A. californicus</i>	Espinha <i>et al.</i> , 1995
1994	EMBRAPA, Sao Paulo, Brazil	contaminant on glasshouse cassava	<i>N. californicus</i>	M. Mebelo – pers. comm.
1994	mainly Mediterranean Portugal	<i>Phaseolus</i> sp.	<i>A. californicus</i>	Ferreira & Carmona, 1994
1994	Lazio, Italy	vineyards	<i>A. californicus</i>	Nicòtina, 1996
1994	Poland	gerbera	<i>A. californicus</i>	Kielkiewicz & Witul, 1995
1995	Bruson, Switzerland	strawberry	<i>N. californicus</i>	Baillod <i>et al.</i> , 1995



Figure 3.1 Records of *N. californicus* from the 1950's and 1960's.



Figure 3.2 Records of *N. californicus*. Previous records are in black, those from the 1970's are in red.



Figure 3.3 Records of *N. californicus*. Previous records are in black, those from the 1980's are in red.



Figure 3.4 Records of *N. californicus*. Previous records are in black, those from the 1990's are in red.



3.2 Occurrence of *N. californicus* in the UK

3.2.1 Introduction

Neoseiulus californicus is not native to the UK. For a short while a licence was granted by DETR for its release as a biocontrol agent, based on a spurious synonymy with *Typhlodromus marinus* (Willmann). Releases occurred from 1993-1995 (Pete Squires, Koppert Ltd – personal communication), then the licence was withdrawn. The aim of the work in this chapter was to investigate the recent occurrence of *N. californicus* in the UK by field collections.

3.2.2 Materials and methods

During 1997 to 2000, from spring to autumn, leaf samples were taken from a range of locations including the West of England, Kent and East Anglia. A range of host plants was sampled: apple, hop (*Humulus* sp.), blackcurrant (*Ribis nigrum*), raspberry (*Rubus idaeus*) and strawberry. The localities were selected on the basis of a prevalence of predatory mites, or where growers or advisors had noted the presence of predatory mites that looked larger and more active than *Typhlodromus pyri* (the native species they are familiar with). During the summer (June to September) of the year stated in the results leaf samples were made. The leaves were randomly selected from throughout the plot examined. Sample size varied but was usually a minimum of 50 leaves. In the autumn of each year, overwintering bands were placed in localities where *N. californicus* had been found during the summer. Overwintering bands are a good means of collecting large numbers of phytoseiid mites collected, as there is often a lack of suitable overwintering sites on plants (Fitzgerald, personal communication). These overwintering bands consisted of strips of hessian or velour (8cm wide), which were wrapped around a branch or stem several times and secured with insulation tape. Overwintering bands of this type have been found to be a convenient means of collecting overwintering mites (Fitzgerald, 1999) and any sheltering arthropods are easily extracted by placing the bands in Tullgren funnels (Burkard Agronomics) for a few days. These overwintering band collections were included in the work on the potential of *N. californicus* to survive the winter (see chapter 4, section 4.4), however the information is included here so it can be related to the summer surveys on leaves.

3.2.3 Results

Three locations with dwarf hops were examined, and the results are presented in Table 3.2. *Neoseiulus californicus* were found in all of them, in low numbers and in conjunction with other phytoseiid species. Table 3.3 shows the results from collections of material from soft fruit. Of the two strawberry plots examined, both had *N. californicus* present and in fairly high numbers (100% and 83% of the phytoseiids collected were *N. californicus*). No *N. californicus* were found on raspberries; *T. pyri* was the predominant phytoseiid at Horticulture Research International (HRI), East Malling (EM). In the blackcurrant plantations, *N. californicus* occurred in two of the three locations. In Ticehurst, Kent, it was present in greater numbers during the summers of 1998 and 1999 (100 and 88% of the mites collected) and then was found overwintering at lower densities (4%). In Chartham, Kent, it was present in 1997, but in subsequent years *T. pyri* was found instead. Of the six apple orchards investigated, only one had *N. californicus* present. The results are presented in Table 3.4. In a similar scenario to the blackcurrant plantation at Ticehurst, during the summer of 1998 all the phytoseiids found were *N. californicus*, but a lower percentage were present in the overwintering bands (13%).

Table 3.2 Mites collected from dwarf hop during 1997-2000. (o/w = overwintering)

location	year	material inspected	species found				Total no. of phytoseiids	No. of <i>N. californicus</i>	% <i>N. californicus</i>
			<i>N. californicus</i>	<i>A. cucumeris</i>	<i>T. pyri</i>	other			
Claston, Hereford	1997	100 leaves					none		
		cones o/w	•	•	•		60	7	12
		leaf litter o/w	•	•		•	202	3	2
		twigs & leaves o/w		•	•		2	0	0
	1999	30 leaves	•				1	1	100
HRI, East Malling, Kent	1997	cones o/w			•		5	0	0
		leaf litter o/w		•	•	•	30	0	0
	1998	cones o/w		•	•		13	0	0
		leaf litter o/w			•	•	57	0	0
	1999	cones o/w	•	•	•	•	69	55	80
	1998/9	58 o/w bands		•	•	•	147	0	0
	1999/2000	36 o/w bands	•	•	•	•	127	8	6
		cones on hedge o/w	•				3	3	100
Ledbury	1999	20 leaves	•				1	1	100

Table 3.3 Mites collected from soft fruit during 1997-2000. (o/w = overwintering)

host plant	location	year	material inspected	species found				Total no. of phytoseiids	No. of <i>N. californicus</i>	% <i>N. californicus</i>
				<i>N. californicus</i>	<i>A. cucumeris</i>	<i>T. pyri</i>	other			
strawberry	HRI, East Malling, Kent	1999	20 leaves	•				15	15	100
	Mockbeggar Fm, Stroud, Kent	1998	88 leaves	•	•		•	40	33	83
raspberry	HRI, East Malling, Kent	1998	20 leaves			•	•	5	0	0
	Mockbeggar Fm, Stroud, Kent	1998	50 leaves					none		
blackcurrant		1998	8 leaves	•				3	3	100
	Ticehurst, Kent	1999	200 leaves	•			•	42	37	88
		1999/2000	66 o/w bands	•	•	•	•	27	1	4
	Upper Horton Fm, Chartham, Kent	1997	512 leaves	•				7	7	100
		1998	118 leaves			•		48	0	0
		1998/9	49 o/w bands			•	•	13	0	0
	Highland Court Fm, Bridge, Kent	1998	50 leaves			•		7	0	0
		1999	50 leaves			•		23	0	0

Table 3.4 Mites collected from apple during 1997-2000. (o/w = overwintering)

location	year	material inspected	species found				Total no. of phytoseiids	No. of <i>N.</i> <i>californicus</i>	% <i>N.</i> <i>californicus</i>
			<i>N.</i> <i>californicus</i>	<i>A.</i> <i>cucumeris</i>	<i>T. pyri</i>	other			
Stoke Fruit Fm, East Anglia	1998	100 leaves	•				6	6	100
	1998/9	60 o/w bands	•	•		•	16	2	13
HRI, East Malling	1998/9	11 o/w bands			•	•	88	0	0
Fern Ditton, Cambridge	2000	10 leaves			•		10	0	0
Tremletts Fm, Bilde, Kent	2000	50 leaves			•		18	0	0
Target Fm, Marden, Kent	2000	50 leaves			•		19	0	0
West Pike Fm, Laddingford, Kent	2000	50 leaves			•		6	0	0

3.3 Discussion

On first glance at Figures 3.1-3.4 it might seem as though *N. californicus* spread from southern Europe and North Africa in the 1950's and 1960's to South America in the 1970's and into North America in the 1980's. However, it is more likely that the initial discoveries of this mite in the 1950's and 60's led to further identifications in the 70's and 80's, rather than it having spread across the Atlantic. It is possible that Figures 3.1-3.4 show an intra-continental movement of mites. However, making a definitive observation of this sort is difficult as the records of *N. californicus* may merely reflect an increasing awareness of the presence of this phytoseiid in fruit plantation fauna. Indeed, it may even reflect a general increase of knowledge and interest in phytoseiid mites from the 1950's (when the first keys were produced) to the present day. It is likely that more records of *N. californicus* are made as researchers become increasingly interested in mite orchard fauna and identification.

It can be noted that *N. californicus* occurs in two geographically separated regions: Europe/North Africa and the Americas. This raises an interesting question; were *N. californicus* moved from one region to the other, perhaps on plant material? Further work, especially genetic studies, could provide an answer. An investigation into the potential existence of different strains, with distinct biological characteristics, of *N. californicus* was carried out and the results are presented in chapter 6.

The field survey of the occurrence of *N. californicus* in the UK showed that it is present in unprotected fruit and hop plantations. *Neoseiulus californicus* had been released in 1996 and 1998 in the dwarf hop plantation at HRI-EM as part of a field trial to control *T. urticae*. No known releases had occurred at any of the other sites. This raises a number of questions: has it spread from other release sites in the UK? If it can survive winter conditions (see chapter 4), was it released for spider mite control in propagation and has since become established on those plants in the field?

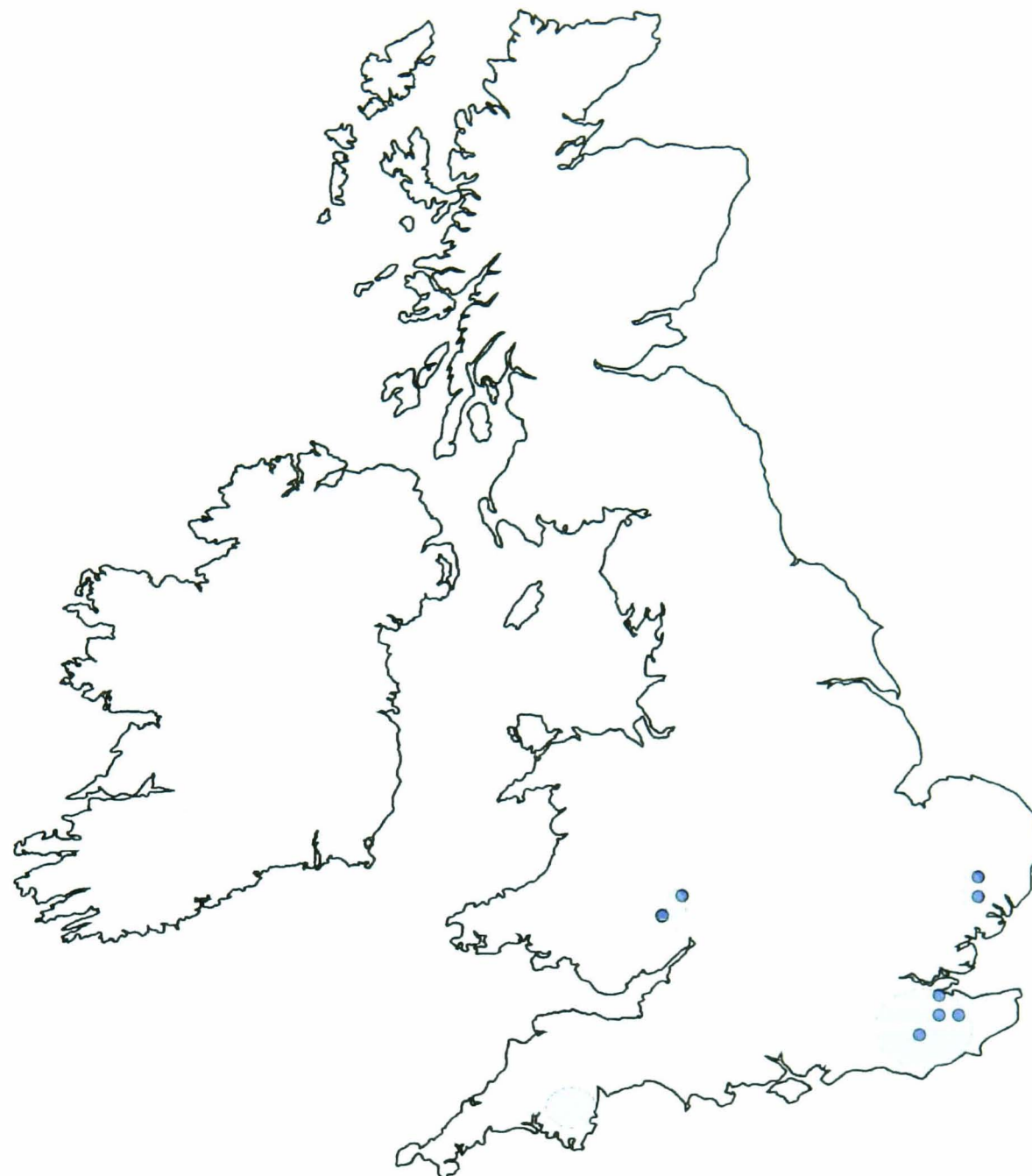
Where *N. californicus* was found in this survey it was often in low numbers and was not the predominant phytoseiid species present. There are several explanations: it may be out-competed

for food or overwintering sites by other mite species, it may be sensitive to certain spray programmes, or there may not have been enough prey present to sustain a large population. In a field experiment, when *N. californicus* was released as a biocontrol agent for *T. urticae* in dwarf hops, the numbers of *N. californicus* dropped in response to a decline in prey numbers (Colin Campbell – personal communication). However, in the present study, other phytoseiid species remained at higher densities and perhaps these (*T. pyri* in particular) are better at exploiting alternative food sources than *N. californicus*. In culture, *T. pyri* can be maintained at high numbers (approximately 150 individuals per culture plate) when fed on *V. faba* pollen alone, whereas *N. californicus* numbers remain low (approximately 30 individuals) and healthy cultures of this mite require a live food source such as *T. urticae* (personal observation). *Typhlodromus pyri* has also been shown to feed on apple powdery mildew (*Podosphaera leucotricha*), and can complete its development when reared on this as a sole food source (Chant, 1959). Another possibility is that *N. californicus* is susceptible to commonly used pesticides and that other phytoseiids found in greater numbers are not. Alternatively, competition between predatory mites might have resulted in lower numbers of *N. californicus*. Palevsky *et al.* (1999) found that selective predation of *N. californicus* eggs was carried out by *Typhlodromus athiasae* Porath and Swirski and that this may prevent *N. californicus* establishing in Israeli apple orchards. A study on egg predation by Schausberger and Croft (1999) found that *Amblyseius cucumeris* (Ouedemans) and *N. californicus* would feed on phytoseiid eggs, with a preference for heterospecific eggs. *Typhlodromus pyri* consumed fewer eggs and appeared to have difficulty in piercing the egg chorion (Schausberger & Croft, 1999). In a comparison of nymphal feeding and predation by *N. californicus* and *N. fallacis* it was found that *N. californicus* displayed a greater amount of intra- and interspecific predation than *N. fallacis* (Monetti & Croft, 1997).

Figure 3.5 is a diagram of the UK showing the regions in which *N. californicus* was released, by Koppert Ltd, and those in which it was found in the current study. No collections were made in Devon, as the releases there were on nursery stock, and there are few commercial dessert-apple growers in that region. During 1998-2000, *N. californicus* was found in areas where it had been released (during 1993-1995) under licence. There were no samples taken from locations where Koppert did not release *N. californicus*; the areas where they made releases correspond to the

main fruit growing regions in the UK. In retrospect it would have been interesting to have searched for suitable locations in other areas of the country and sampled there also. As collections were not made from every fruit plantation throughout the UK, it is not possible to comment on the UK-wide prevalence or spread.

In summary, *N. californicus* is not native in the UK. It was released between 1993 and 1995 and was still present in 2000. It has been found to survive winter field conditions and pesticide applications and has become part of the phytoseiid fauna in fruit plantations in the UK.



- regions where *N. californicus* has been known to be released (Pete Squires, Koppert UK Ltd – personal communication)
- areas where *N. californicus* was found

Figure 3.5 Outline of the UK showing regions in which *N. californicus* has been known to be released (previous to 1997) and areas in which it was found during 1997-2000.

CHAPTER 4: WINTER SURVIVAL

4.1 Introduction

There have been various studies on overwintering in phytoseiid mites. Many female phytoseiid mites undergo facultative reproductive diapause. The most discernible difference between diapausing and non-diapausing females is that during diapause they do not lay eggs, and normally diapause can only occur in adult females after mating. They also appear paler, flatter and less active (Overmeer, 1985a; Veerman, 1992). The contents of the body cavity of diapausing mites are often granular in appearance, which may be due to accumulation of lipids as energy reserves (Morewood & Gilkeson, 1991). Shelter-seeking and migration may be diapause-related behaviours and *Amblyseius* species prefer low herbaceous vegetation or soil litter for overwintering (Veerman, 1992). Diapausing females may be less sensitive to pesticides than non-diapausing females and have greater longevity (Veerman, 1992).

In colder climates only diapausing females overwinter, males and juveniles die. Some species lack a diapause response altogether, and overwinter without diapausing (Overmeer, 1985a). Many phytoseiid mites can only undergo diapause if they have been exposed to diapause-inducing conditions during juvenile development (Veerman, 1992). However, some species can 'switch' into diapause as adults, having been reared in non diapause-inducing conditions (Van Houten, 1989). In most mites (that undergo diapause) induction takes place during the developmental stages prior to the one in which it is expressed (Veerman, 1992). For *Amblyseius cucumeris* the highest incidence of diapause was seen when exposure to diapause-inducing conditions began before the eggs hatched, implying that late embryonic stages of this mite are sensitive to photoperiod (Morewood & Gilkeson, 1991). Van Houten and Veenendaal (1990) showed that all stages of *Amblyseius potentillae*, from late embryonic onwards, were sensitive to photoperiod in varying degrees, and sensitivity was greatest for protonymphs.

Photoperiodic diapause induction has been demonstrated for 15 species of phytoseiids. In all instances the response has been the 'long day' type, where diapause is induced by relatively short daylengths (Veerman, 1992). Reported critical photoperiods for phytoseiid mites range from 11.2h to 15.3h and vary amongst species and populations (Croft, 1971). The critical daylength (that at which 50% of diapause occurs) for *T. pyri* is 12.5 to 13.5h (Fitzgerald &

Solomon, 1991). For *A. potentillae* it is 14.5h (Van Houten & Veenendaal, 1990) and for *A. cucumeris* it is 12.45h (Morewood & Gilkeson, 1991). There is a relationship between latitude and critical daylength for inducing diapause (Overmeer, 1985a). Some phytoseiid species, with a wide geographical distribution, have diapausing populations at higher latitudes and non-diapausing ones at lower latitudes, or in warmer areas (Veerman, 1992). The critical daylength for *Typhlodromus occidentalis* Nesbitt, in USA, increases from south to north by 0.56-1.04h with every 5° change in latitude (Croft, 1971).

Temperature may also have an effect on the critical daylength. At high temperatures diapause is prevented (Overmeer, 1985a). Thermoperiodic diapause induction has been demonstrated in *A. andersoni* and *A. cucumeris* when kept in constant darkness, however a similar effect could not be demonstrated with the tetranychid mite *Tetranychus urticae* (Veerman, 1994). Temperature can modify photoperiodic response and may act independently as a stimulus for diapause induction (Veerman, 1992). Night-time temperature may be more important than day-time temperature; for *A. potentillae*, diapause was only induced when the cool phase of the thermoperiod coincided with the scotophase (Van Houten *et al.*, 1988). Relative humidity can also affect diapause. Diapause duration for *A. potentillae* was shortest when exposed to low RH (35%) during diapause induction and high RH (75%) during diapause termination (and longest under reverse conditions) (Van Houten & Veenendaal, 1990).

Ability to diapause may depend on β -carotene in the diet. Broad bean pollen (*Vicia faba*) lacks β -carotene, whereas *Mesembryanthemum* sp. (iceplant) pollen does not (Overmeer, 1985a). *Dorotheanthus bellidiformis* (Livingstone daisy) has thirty times more β -carotene than broad bean (Veerman, 1994). Vitamin A (or a derivative) has to be present in the diet of *Amblyseius andersoni* whilst it is experiencing short-day cycles for diapause to be induced (Veerman, 1994). Veerman (1994) hypothesises that vitamin A, or its derivative, is the photoreceptor pigment for the photoperiodic clock in *A. andersoni*. Van der Geest *et al.* (1991) found that there was no difference in cold hardiness between female mites fed on *Mesembryanthemum* pollen compared with those fed on *V. faba* pollen, provided the *V. faba* pollen was supplemented with β -carotene. β -carotene is a necessary factor in the physiological mechanism of photoperiodic induction of *A. potentillae*, and not for the expression of the diapause response (Van Houten & Veenendaal, 1990). However, this is not

the case for all mite species; there were no significant differences in the ability to diapause between *Typhlodromus pyri* fed on *V. faba* pollen, *T. urticae*, or *Panonychus ulmi* (Fitzgerald & Solomon, 1991). Although *A. potentillae* and *A. cucumeris* do not enter diapause if fed solely on *V. faba* pollen, *T. pyri* does (Overmeer *et al.*, 1989).

Diapause cannot be stopped, once it has begun, simply by transferring the organism to conditions favourable for growth and/or reproduction. 'Diapause development' has to take place first (Veerman, 1992). Duration and 'depth' of diapause, and response to external factors, are species specific or strain specific characteristics (Tauber *et al.*, 1986). The rate of diapause development is influenced by photoperiod and temperature. Diapause depth gradually diminishes during late autumn and winter (Veerman, 1992). There may be a 'post diapause transitional phase of dormancy' once diapause has ended, during which quiescence and/or development may occur resulting in a loss of diapause characteristics (Tauber *et al.*, 1986).

There has been little published on overwintering, diapause and cold hardiness of *Neoseiulus californicus*. Female *N. californicus*, in southern France, were found searching for prey on apple trees and in ground cover as late as mid-November and at least as early as the end of February; no immatures or males were observed, which indicated a reproductive diapause (Raworth *et al.*, 1994). *Neoseiulus californicus* could commence reproductive activity at any time during the winter, with the natural light regime, if given adequate temperature and food. At 17-20°C and fed on a diet of *T. urticae* the females laid their first egg in 7.9 days, with all but one (of 39 individuals) producing viable progeny (Raworth *et al.*, 1994). In Uruguay apple orchards, *N. californicus* overwinters as adult females, and the preferred site was found to be in the bark of the tree trunk or in the buds (Bruhn & Beltrame, 1981).

Castagnoli *et al.* (1996) investigated diapause induction in *N. californicus* from Italy. The phytoseiids had been mass-reared at 25°C (L:D 16:8, 80±10% RH) for many years. At 21°C, L:D 10:14, 82% went into diapause. They found that daylength affected diapause induction to a greater extent than temperature. Castagnoli *et al.* (1996) found little difference in percentage of individuals diapausing when they were fed either *T. urticae* or *Quercus* sp. pollen as a food source. They postulated that a diet solely consisting of *Quercus* sp. pollen provides enough carotenoids to allow diapause to occur; notably, they also induced diapause

in *A. cucumeris* (which is unable to diapause if fed *V. faba* pollen (Overmeer *et al.*, 1989)). In addition, Castagnoli *et al.* (1996) noted that the time necessary for diapause termination in *N. californicus* was 3.4 ± 0.9 days at 21°C.

The aim of this chapter was to investigate the diapause ability and low temperature survival potential of *N. californicus*, as these characteristics are important factors when considering the biocontrol potential of a predatory mite in the UK. If, like *Phytoseiulus persimilis*, it is unable to survive the winter then it would have to be re-released each year. However, if it is able to overwinter successfully, there is the potential for a population to survive and establish from one year to the next. This is particularly useful for a perennial crop such as apples.

4.2 Ability to diapause

During winter conditions, temperate phytoseiid mites generally undergo reproductive diapause; females cease laying eggs and become less active (Overmeer, 1985a). An investigation into the ability to diapause was carried out for three strains of *N. californicus*: from Spain (supplied by Koppert Ltd, Netherlands), the USA (supplied by Nature's Control) and the UK (collected from a strawberry plot at HRI-East Malling). A strain of *T. pyri* that was known to diapause was used as a control.

Several factors had to be taken into account. Day length, rather than temperature, has been shown to be the factor influencing diapause induction in *N. californicus* (Castagnoli *et al.*, 1996) and the critical photoperiod for various phytoseiid mites that are mentioned in the literature ranges from 10 to 14.5h (see Table 4.1). It was important to supply live food as a source of β -carotene is necessary for some species to diapause (Overmeer, 1985a). Most phytoseiid mites will only undergo diapause if their juvenile stages have experienced diapause-inducing conditions (Veerman, 1992), therefore the individuals had to be reared from the egg stage through to adult in short daylengths. Castagnoli *et al.* (1996) found that 3.4 ± 0.9 days at 25°C were necessary for termination of diapause in individuals that had undergone diapause at 21°C, 10L:14D, therefore if egg laying resumed after a time lag of 4 days, then diapause was assumed to have definitely taken place.

Table 4.1. The critical photoperiod of a range of phytoseiid mites.

species	critical photoperiod (h)	reference
<i>T. pyri</i>	12.5 - 13.5	Fitzgerald & Solomon (1991)
<i>T. occidentalis</i>	10 – 12	Hoy & Flaherty (1975); James (1988)
<i>A. cucumeris</i>	12.45	Morewood & Gilkeson (1991)
<i>A. potentillae</i>	14.5	Van Houten & Veenendaal (1990)

4.2.1 Materials and methods

Thirty gravid females of each strain were placed on culture plates (as described by Overmeer (1985b)) to lay eggs (see Chapter 5, Figure 5.1), and the culture plate maintained in a CT room at $21\pm1^{\circ}\text{C}$ with a short day length (8L:16D). This light regime was selected so as to encompass the possible critical daylength within a large margin. All the mites were fed on *T. urticae*. Eggs were taken and placed, one on each square, on a culture plate divided up into 18 with strips of filter paper (see Chapter 5, Figure 5.2). The eggs were allowed to develop to adult, females mated with males from the same strain, and each female examined every day for egg laying. Females which had mated and did not lay eggs for two weeks were deemed to be diapausing. This was confirmed by moving the females into a CT room at 21°C 16L:8D and monitoring every day for egg laying. The time taken from exposure to long daylength (16L:8D) to oviposition of the first egg was recorded. Data for between 19 and 34 individuals of each mite species/strain were collected. The eggs laid by the Spanish non-diapausing females (as none of these were found to diapause) were placed onto individual arenas, allowed to develop to adult and the resulting females mated. Thus, a second generation were examined. The data were compared by a chi-squared test.

4.2.2 Results

All the *T. pyri* individuals, and 96% of the UK strain of *N. californicus*, underwent diapause (Table 4.2). However, only a few individuals from the American strain and none of the Spanish strain were able to diapause. No diapausing individuals in the second generation of Spanish mites were found. The results were compared by a chi-squared test and were significant ($P<0.001$).

The mean time taken from exposure to long daylength (16L:8D) to oviposition of the first egg was 8.74 days for *N. californicus*, with a maximum time of 14.03 days and a minimum of 6.21 days recorded. For *T. pyri* the mean time was 23.62 days, with a maximum of 30.17 and a minimum of 9.03 days. All the females transferred to long daylengths resumed oviposition.

Table 4.2. The percentage of individuals of *T. pyri* and three strains of *N. californicus* that underwent diapause in short day conditions (8L:16D, 21±1°C).

	<i>N. californicus</i>				
	<i>T. pyri</i>	UK strain	Spanish strain	Spanish strain 2 nd generation	USA strain
% of individuals in diapause	100	95.7	0	0	16.1
n	19	23	34	19	31

$$\chi^2 = 103.52 \text{ df} = 4 \text{ } P < 0.001$$

4.2.3 Discussion

This work has shown that *N. californicus* has the potential ability to diapause. Therefore there are implications for future policy on introductions of this species in the UK as a biocontrol agent. There was different diapause potential exhibited by mites of different origins. The Spanish *N. californicus* did not enter diapause when exposed to short daylengths, nor did a second generation. This indicates that potential for diapause is not triggered after exposure of more than one generation to short daylengths (as may have been the case with the UK mites), and that it is probably an inherent trait and linked to a genetic predisposition. The possibility that a few Spanish *N. californicus* individuals may be able to diapause in the UK cannot be discounted. It is possible that exposure in the field would trigger diapause in a few individuals and further work investigating the differences in diapause in these strains and the ease with which it is possible to manipulate these differences would be necessary before making further statements on the diapause characteristics of these strains. It is possible that a population of Spanish mites would be less-able to survive UK winter field conditions than an American one.

It should be noted that the use of a 8L:16D light regime may limit the potential of a species to diapause. If the light regime used is one where the light period falls a long way below the critical photoperiod, then some species will not enter diapause at all. It is possible that this was the case for the Spanish *N. californicus*.

There are a couple of factors influencing why the mites from different geographical origins display differences in diapause propensity. A necessary factor is genetic influence. Morewood and Gilkeson (1991) found strain differences in *A. cucumeris* when examining its diapause ability. They selected for non-diapause and obtained a reduction in the percentage population diapausing from 88% to 33% over 14 generations. Van Houten *et al.* (1995) were able to select for non-diapause in both *A. cucumeris* (41% to 0% in ten generations) and *Amblyseius barkeri* (Hughes) (67% to 0% in six generations). For these species there is evidently a genetic influence on diapause ability, and this trait is fluid and manipulatable from one generation to the next. Hence it is probable that any *N. californicus* released in the UK may have the potential to diapause, although their success may vary from strain to strain.

Diapause is an advantage in that it safeguards mites from a response to start laying eggs if a few warm days are experienced during winter. It may also trigger physiological effects which enhance survival potential. Some species have a genetic potential to diapause and in regions where this would be advantageous that species tends to exhibit diapause. Thus such species would be influenced by geographical origin, and there has been shown to be an effect of both latitude and altitude on diapause ability and also critical daylength required to induce diapause. Some phytoseiid species with a wide geographical distribution, have diapausing populations at higher latitudes and non-diapausing ones at lower latitudes, or in warmer areas (Veerman, 1992). It is reasonable to assume that mites would evolve to respond to the critical daylength appropriate to where they occur. Bondarenko and Hai-Yuan (1958) examined the critical daylengths for *T. urticae* and found decreasing latitude corresponded to a decrease in critical daylength (60°, 45° and 41°N with 17h, 12.5h and 12h respectively). Croft's (1971) study of *T. occidentalis* found both latitude and altitude to have an effect with similar trends to the afore-mentioned *T. urticae* experiment (47.5° + 670ft, 40.3° + 4500ft, 34° + 4800ft and 34° + 1100ft with 15.4h, 14.6h, 13.8h and 13.2h respectively). Unfortunately these papers did not include data for percentage of individuals diapausing though Croft (1971) states that it was >90% for each strain. The latitudes corresponding to the origin of the strains of *N.*

californicus in the current study are 51.3° (Kent, UK), 39.5° (Valencia, Spain) and 34-38° (California, USA). The percentage of individuals in diapause at the greater latitude, the UK, was 96%. From the lower latitudes, Spain and the USA, the percentage of diapausing individuals was less, 0 and 16% respectively.

The ability to diapause appears to be a requirement for successful overwintering in the UK. As far as it has been possible to establish the origins of the *N. californicus* introduced into the UK in earlier years, it appears that they were sourced from both Spain and the USA. In the present study, *N. californicus* from Spain did not enter diapause when exposed to short day length. A proportion (16%) of the *N. californicus* originating from the USA did enter diapause, indicating that the gene pool includes a greater genetic predisposition for diapause. On the basis of this initial investigation, it appears likely that the *N. californicus* found to be surviving in the UK may have originated from mites from the USA rather than the Spain. Further work to examine the genetic relatedness of these mites may elucidate the origin of the UK strain and show whether they are indeed of American origin.

4.3 Low temperature survival

4.3.1 Introduction

Studies on the relationship between cold hardiness and diapause in phytoseiid mites have demonstrated that either may occur independently of the other. In some cases cold hardiness is a component of, or may be enhanced by, diapause (Morewood, 1993). Cold hardiness is greater in diapausing females (shown to be the case for *Amblyseius umbraticus* (Chant) and *Phytoseius finitimus* Ribaga), thus females in diapause are better adapted to winter conditions than non-diapausing mites (Overmeer, 1985a).

There have been two approaches to studying cold hardiness of phytoseiid mites. One approach has been to determine their supercooling points (SCP), i.e. the lowest temperature that can be reached before body fluids freeze. The other approach has been to describe cold hardiness in terms of the number of days of exposure at a certain temperature that results in 50% mortality (LT₅₀). Bale and Walters (1997) suggest a third approach. A lethal time (LTime) analysis is appropriate for chill tolerant species, to quantify their cold hardiness. However, for chill susceptible species a lethal temperature (LTemp) approach would be

appropriate, as mortality occurs in response to brief exposures to low temperatures. A combination of SCP, LTemp and LTime data would illustrate the cold tolerance of a particular species and would provide a basis for predicting the response of a population to low temperatures in field situations (Bale & Walters, 1997).

There is a problem associated with the Bale and Walters (1997) approach; the numbers of individuals needed for the LTime and LTemp analyses is very large (>150). Ideally these experiments would be carried out on females both diapausing and non-diapausing. It would also be interesting to examine effects of acclimation regimes. However, due to constraints of time and an inability to culture the large numbers of *N. californicus* required for these experiments, it was decided to concentrate on a SCP analysis of *N. californicus* and a truncated version of the LTime and LTemp analyses.

4.3.2 Supercooling point

The SCP is the temperature at which the fluid contents of the body freezes. Small volumes of water do not freeze at around 0°C, instead they supercool and remain liquid until they reach temperatures much lower than the melting point (Lee, 1991). The SCP of the mites was measured using a differential scanning calorimeter (DSC). When a substance changes state, e.g. from a liquid to a solid, heat energy is either released or absorbed (in the case of freezing latent heat of fusion is given out). Differential thermal energy analysis (DTA) measures the energy difference between a sample and a reference material. Block (1994) reviewed the uses of DSC for studying cold resistance in invertebrates, and further detail concerning calibration and the potential of DSC in ecophysiological research can be found therein.

4.3.2.1 Materials and Methods

The DSC used was a Mettler Toledo DSC820 with mechanical intra-cooler, able to go down to -80°C. This DSC uses the 'Boersma' DTA system, where the sample (S) and reference (R) are heated by a single heat source (see Figure 4.1). The temperatures are measured by sensors attached to the pans that hold the material. The mites used for the DSC study were adult females of 3 strains of *N. californicus* (from the UK, America and Spain), *T. pyri* and *P. persimilis*. For the *N. californicus* and *T. pyri* a comparison was made between those that had been kept in culture in long daylength (21°C, 16L:8D) and those that had been kept in diapause-inducing conditions for several weeks (21°C, 8L:16D). For each variable considered

(diapausing vs. non-diapausing and/or mite species) approximately ten individuals were used. An empty 40 μ l aluminium crucible (base and lid) was weighed. The mites to be analysed were placed in the crucible, the lid crimped shut, and the crucible re-weighed. A maximum of four mites at a time could be analysed, when more than this was attempted it was found impossible to contain them. The data for the sample (weight, number of individuals, sample origin) was entered into the computer, the sample placed into the DSC and the programme started. The thermal programme was set to cool to 0°C at a rate of 2 degrees min⁻¹, then cool at a rate of 1 degree min⁻¹ to -30°C. The temperature was then brought back up at a rate of 20 degrees min⁻¹ as the melting of the sample was not of consideration in this instance. The sampling interval was 1s. The resulting plot or ‘thermograph’ of heat flow (mW) against time (min) was displayed on a computer and evaluated using the STAR^e software (version 6.1). Each individual gave a separate peak when the SCP was reached; thus more than one individual could be analysed at the same time. If the SCP of more than one individual had been identical then a larger or ‘double’ peak would have been visible. Once the programme had finished, the crucible was removed from the DSC and opened to confirm the number of individuals inside matched the number of SCP peaks observed, and to establish whether the mites survived.

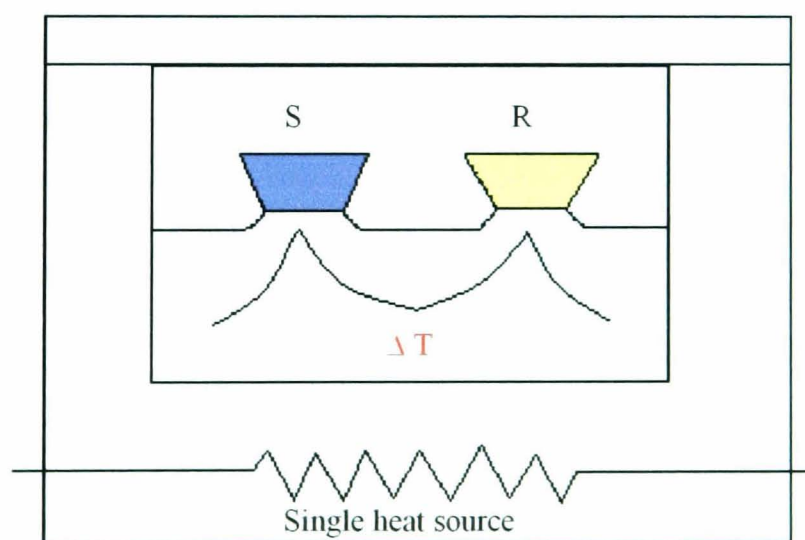


Figure 4.1. Diagram of the thermal analysis system used in ‘Boersma’ DTA (McNaughton & Mortimer, 1975).

4.3.2.2 Results

None of the mites survived freezing; all were dead after the experiment. An example of the trace obtained from the DSC can be seen in Appendix II. The mean SCP values for the mites tested are shown in Table 4.3.

Table 4.3. Mean SCP values for different mite species, reared in conditions of either long daylength (LD) (21°C, 16L:8D) or short daylength (SD) (21°C, 8L:16D).

species	origin	rearing conditions	n	mean weight (µg)	mean SCP (°C) ± st dev	range	
						min	max
<i>P. persimilis</i>	Koppert UK Ltd	LD	8	9.5	-22.32 ± 1.16	-20.44	-24.33
<i>T. pyri</i>	Kent, UK	LD	11	7.6	-25.40 ± 1.74	-22.89	-28.62
		SD	11	3.4	-25.53 ± 0.72	-24.90	-27.48
<i>N. californicus</i>	Valencia, Spain	LD	10	6.2	-23.35 ± 1.64	-19.66	-25.24
		SD	11	6.5	-22.83 ± 1.27	-20.74	-24.73
	California, USA	LD	11	7.1	-21.77 ± 1.54	-19.12	-24.17
		SD	8	4.0	-22.36 ± 1.82	-19.12	-24.32
	Kent, UK	LD	12	10.4	-19.89 ± 1.50	-18.37	-24.13
		SD	9	13.3	-22.47 ± 1.44	-21.31	-24.98

4.3.3 Lethal Temperature

4.3.3.1 Materials and Methods

An incubator (Low Temperature Incubator Mk4, Astell Hearson) was set to run at -10°C (as this temperature is about 10 degrees above the mean SCP) and allowed to stabilise over several days. A thermometer was used to record the temperature within the incubator. Mites were taken from a diapausing *N. californicus* culture (US strain) and were placed in small polyethylene capsules with hinged lids (BEEM capsules, size 3, Agar Scientific), 5 mites per capsule. The capsules contained a small rectangle of filter paper to ensure moisture did not build up within them. The mites were placed onto this paper so that they were not in full contact with the tube and thus would not stick and freeze to any moisture on the surface as it cooled, as this would affect their survival. The BEEM capsules are ideally suited to this purpose as the lids fit over the outside of the tube, reducing the risk of squashing the highly active mites on closure (Eppendorfs could be used but there is a possibility of squashing the

mites in between the lid and the side of the vessel). Five capsules (i.e. twenty-five mites) were placed into a boiling tube and it was placed in antifreeze in the incubator for one hour, after which time it was removed and allowed to warm up to room temperature. Mortality of the mites was then assessed by examining them within the capsules.

4.3.3.2 Results

All the *N. californicus* tested survived exposure for one hour at -10°C i.e. survival was 100%. When they were examined after exposure, all the mites were running around the inside of the capsules and appeared unaffected.

4.3.4 Lethal Time

4.3.4.1 Materials and Methods

An incubator (Low Temperature Incubator Mk4, Astell Hearson) was set to run at -7°C and allowed to stabilise. A temperature logger (Tinytag Plus, Gemini Data Loggers: Temperature range H ($-30 \rightarrow +50^{\circ}\text{C}$) & range G Standard probe ($-40 \rightarrow +125^{\circ}\text{C}$)) was used to record the temperature within the incubator. The mites used were: *N. californicus* Spanish, American and UK strains and *T. pyri*. Mites were either taken from culture plates that had been kept for a few weeks in long daylength conditions (LD) of 21°C and 16L:8D, or from diapausing cultures that had been kept in short daylength conditions (SD) of 21°C and 8L:16D. For each group (mite type and either SD or LD) the following procedure was used: 60 adult females were taken and placed into small polyethylene capsules (BEEM capsules, size 3, Agar Scientific) containing a small rectangle of filter paper, 5 mites per capsule. Two capsules (i.e. 10 mites) were placed into a boiling tube, the remaining space filled with cotton wool and the tube sealed with aluminium foil. This was repeated for all the capsules such that there were six boiling tubes in all. The tubes were labelled 'control', '1', '2', '4', '6', '8'; to correspond with the number of days at which they would be held at -7°C . The probe from the data logger was placed inside one of the tubes that would remain in the incubator for the longest period of time (i.e. 8 days). All the tubes were placed in a water bath at 10°C for 30 minutes, and then in ice for another 30 minutes. This was the same method as used by Bale and Walters (1997) to eliminate possible cold-shock effects. All the boiling tubes, except those labelled 'control', were then placed in a test tube rack that was sitting in a container of antifreeze within the incubator at -7°C . A culture plate was prepared, the capsules were removed from the

‘control’ tubes and carefully opened and left lying on the culture plate. Pollen and live *T. urticae* were made available and the plate was left in a CT room at 21°C, 16L:8D for 24 hours, after which time mortality was assessed. The boiling tubes were removed after 1, 2, 4, 6 and 8 days at –7°C, in correspondence with their labels, and this procedure (placing the capsules on a culture plate with food and assessment 24h later) was followed for all of them.

4.3.4.2 Results

The temperature within the incubator fluctuated by only 0.5°C at the most. After less than 4 days exposure, the mites were still observed to be very active (moving around the culture plates, feeding and laying eggs) when assessed 24h later. From 4 days onwards, any mites observed to be alive had remained in the capsules and, whilst motion of the legs and mouthparts was observed, were seemingly moribund. The exception to this was diapausing *T. pyri* which after 6 days exposure at –7°C, was still very active. The results are shown in Table 4.4. There was some survival after 8 days of exposure: 40% non-diapausing *T. pyri*, 30% diapausing *T. pyri* and 10% UK *N. californicus* from short daylength conditions survived. There was no control for the SD mites, and only some exposure times as there were few individuals available for carrying out the experiment.

Table 4.4. Percentage survival of phytoseiid mites after varying lengths of exposure at -7°C . Mites were reared in either long daylength (LD) or short daylength (SD) conditions.

		% survival after x days exposure					
		control	1	2	4	6	8
LD (1 st run)	<i>N. californicus</i> , USA	100	100	86	75	22	0
	<i>N. californicus</i> , UK	100	100	100	0	20	0
	<i>P. persimilis</i>	100	60	0	0	0	0
LD (2 nd run)	<i>N. californicus</i> , USA	100	70	90	60	30	0
	<i>N. californicus</i> , Spain	100	100	70	33	0	0
	<i>N. californicus</i> , UK	100	100	80	50	20	0
	<i>T. pyri</i>	100	40	70	80	90	40
SD	<i>N. californicus</i> , USA	---	---	---	50	70	0
	<i>N. californicus</i> , Spain	---	---	---	---	60	0
	<i>N. californicus</i> , UK	---	---	---	---	50	10
	<i>T. pyri</i>	---	---	---	---	80	30

4.3.5 Discussion

Supercooling is a complicated subject, with many factors potentially affecting supercooling capacity including ice-nucleating proteins or bacteria, antifreeze proteins and presence of food (Lee, 1991). The SCP defines the point at which the body fluids freeze and therefore the absolute lower limit of survival, as death can occur at temperatures above this point. The supercooling points of the mites studied ranged from approximately -18°C to -28°C . This fits into previously recorded supercooling points for phytoseiid mites, for example, the mean supercooling points of *A. cucumeris* and *P. persimilis* ranged from -19.4°C to -27.1°C (Morewood, 1993). Studies of water droplets have shown that there is an inverse relationship between sample volume and capacity for supercooling (Lee, 1991). In addition, the presence of nucleating agents in liquid samples will cause higher freezing points to occur. The larger a volume of water, the greater the potential nucleators and therefore the higher mean temperature at which it will freeze (Salt, 1966). It would, therefore, be expected that the SCP

of *P. persimilis* would be higher (i.e. warmer) than the other mites studied, as it is a larger mite (greater volume) and also does not undergo diapause as it originates from a warmer climate. It is interesting that this was not found to be the case. The mean SCP recorded for *P. persimilis* in the present study was -22.32°C . Morewood (1992) found the mean SCP of *P. persimilis* to be -22.5°C compared with -20.7°C for *A. cucumeris*, though he notes that there may not be any evolutionary significance for this difference.

In the current study the lowest mean SCP was recorded from *T. pyri*, a native predatory phytoseiid that overwinters successfully in the UK and displayed 100% diapause ability in the previous experiment. However, there are few notable differences between the mites reared in short daylengths compared to long daylengths, as their SCP varies by only one or two degrees. It is probable that the SCPs of a strain reared under certain conditions would fall under a bell-shaped curve such that the mean would correspond to the highest point of the curve, and that some individuals would be at outlying points (see Figure 4.2). In this case ten individuals is too few to make statistical comparisons. It would have been interesting to gather further data, however given the limited supply of mites available it was not possible to do so in this study.

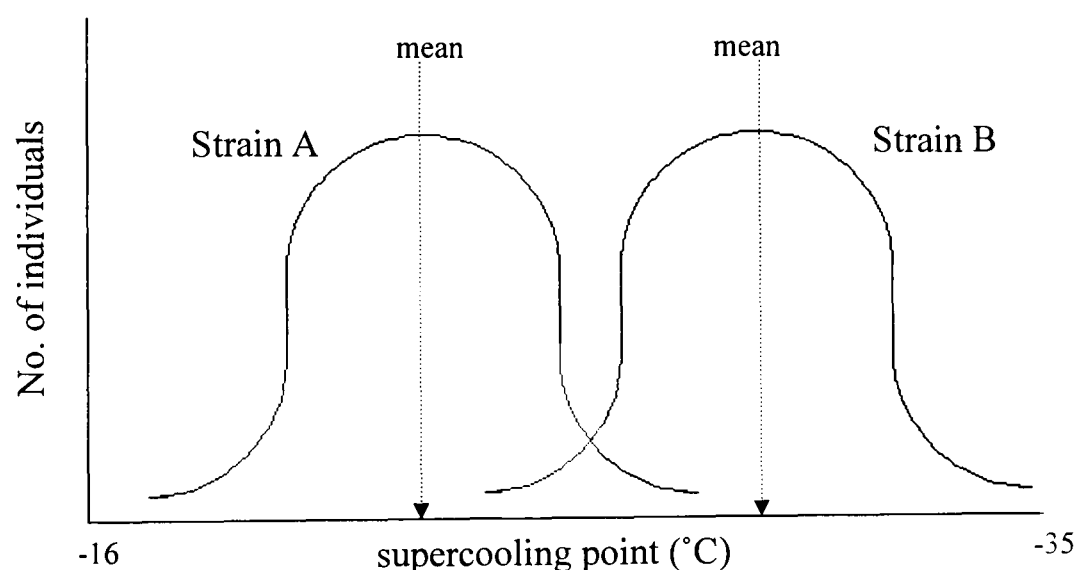


Figure 4.2 Theoretical diagram of supercooling point distribution of a mite.

It should be noted that an acclimation regime, where the mites were held at a lower temperature (e.g. 10°C) for a few days prior to the study, may have had an effect on the SCP; lowering the mean values (Bale – personal communication). However, Morewood (1992) found that feeding status, diapause and acclimation at low temperatures had little effect on the SCP of *P. persimilis* and *A. cucumeris* (measured with a thermocouple, sample sizes of approximately 25 individuals). The SCPs of the phytoseiid mites mentioned here are comparable to other small arthropods that have a liquid diet, one example of which are aphids. For example, the grain aphid *Sitobion avenae* (Fabricius) will supercool to below -20°C and neither acclimation nor starvation improved supercooling capability (Knight & Bale, 1986). In larger arthropods food has been shown to act as a nucleating agent and there is a relationship between gut contents and the ability to supercool, and some species of insect evacuate their guts when exposure to low temperatures occurs (Sømme, 1982). It is possible that the liquid diet of small arthropods, such as aphids and mites, does not have the same effect on supercooling.

In the lethal temperature experiments all 25 *N. californicus* from the SD culture (US strain) survived exposure at -10°C with no previous acclimation regime. Therefore it can be assumed that, for this species, death occurs either above or at the SCP (as all the mites were dead by the end of the SCP experiment), but below -10°C for brief exposure times. Whilst it was noted that the mites were feeding and active after the LTemp experiment, they were not examined subsequently for any effect of low temperature exposure on longevity or fecundity. Morewood (1993) found that brief exposure to -15°C had an adverse effect on the fecundity of *A. cucumeris*, but not on that of *P. persimilis*, suggesting that *A. cucumeris* are susceptible to chilling injury. Morewood (1993) found that acclimatisation at 6 to 7°C, for a week prior to exposure to subzero temperatures, enhanced survival of *A. cucumeris* and *P. persimilis*. Acclimatisation also has a favourable effect on the cold hardiness of *A. potentillae*: prolonged acclimatisation at 4°C produced a higher survival of mites when exposed to sub-zero temperatures (Van der Geest *et al.*, 1991). Future studies on cold tolerance of this species should include these considerations.

As mortality does not occur with brief exposure to low temperatures, it is reasonable to assume that *N. californicus* is not ‘chill susceptible’. An attempt at quantifying cold

hardiness, therefore, was made by carrying out a LTime comparison. It can be seen from the results in Table 4.4 that survival time was shorter for chill susceptible species such as *P. persimilis*; none of the individuals survived longer than one day at -7°C . *Typhlodromus pyri* was the most 'chill tolerant' species, with 30-40% survival after 8 days exposure at -7°C . Approximately 20-30% of *N. californicus* reared in LD conditions survived 6 days exposure and all were dead after 8 days. Notably, chill tolerance increased when the mites were reared in SD conditions with 50-70% survival obtained after 6 days exposure. Van der Geest *et al.* (1991) determined the LT_{50} at -5°C for *A. andersoni*. The LT_{50} value for diapausing *A. andersoni* was three times that of non-diapausing ones, when neither group had been acclimatised to low temperatures. Survival times increased when the mites had been acclimatised to 4°C prior to exposure, though the effect was less for non-diapausing mites. In the current study the USA strain of *N. californicus* appears slightly more tolerant than the other strains. However, as the number of individuals used was low no valid statistical comparison can be made. Approximately 250-300 individuals, preferably at the same developmental stage, are needed for statistical analysis of this experiment (Bale – personal communication), and it was impossible to produce this number of female mites of the same age at the same time. Mass-rearing techniques need to be improved before attempting such a large-scale investigation into cold tolerance of *N. californicus*.

Bale (1996) proposed a new way of categorising cold-hardy insects. The categories were 'freeze tolerant, freeze avoiding, chill tolerant, chill susceptible and opportunistic survival': (i) Freeze tolerant organisms would be found in harshest climates. They synthesise ice nucleating agents in autumn to promote freezing in the haemolymph, water then moves out of the cells to establish osmotic equilibrium, leaving the cell fluids concentrated and unfrozen. These organisms may contain polyols which enable supercooling and protect partially frozen tissues, and antifreeze proteins. (ii) Freeze avoidance organisms usually avoid freezing by extensive supercooling, involving polyols and antifreeze proteins, and the insect can survive in a supercooled state for long periods of time. In these circumstances SCP is a reliable indication of cold hardiness. (iii) Chill tolerant insects have a high level of cold tolerance but some mortality above the SCP, usually due to 'cryoinjuries'. (iv) Chill susceptible species can survive at 0 to 5°C , but at -5°C to -15°C death occurs after brief exposure. Cold tolerance and winter mortality is unrelated to SCP for these insects.

(v) Opportunistic survival applies to 'non-hardy' species that cannot survive below the threshold temperature for normal metabolism, and survival depends on finding favourable overwintering sites. Under this system of categorisation *N. californicus* and *T. pyri* may be considered chill tolerant arthropods, having a reasonably high level of cold tolerance but dying before the SCP is reached.

In summary, the SCP of *N. californicus* was approximately -20 to -23°C , its survival on brief exposure to -10°C was 100% and with long term exposure to -7°C 50% were still alive after 4 days. Rearing in SD conditions increased this survival time. These results indicate that *N. californicus* is a chill tolerant species.

4.4 Field collections

During the survey of the occurrence of *N. californicus* in the UK (see Chapter 3, section 3.2) several sites were identified as having fairly large numbers of *N. californicus* present. These seemed appropriate sites for carrying out a study of the overwintering potential of *N. californicus* in the field. The aim of these experiments was to determine whether the mite could survive our winter field conditions, and whether the style of refuge had an effect on the numbers found therein. As a preliminary investigation, tests were conducted to optimise the Tullgren funnel extraction technique for separating the mites from the overwintering bands.

4.4.1 Optimising the Tullgren funnel mite-extraction technique

An investigation was carried out into the length of time that bands must remain in Tullgren funnels for efficient extraction of phytoseiid mites. There has been no previously published information on this, although Greatorex (1997) notes that few mites were extracted after five days. Given the quantity of bands to be collected during the course of this project, it seemed sensible to establish the minimum amount of time that bands could be left in the funnels.

4.4.1.1 Materials and Methods

Overwintering bands of either hessian (H) or velour (V) were placed around either the trunk (T) or branch (B) of trees in an apple orchard (at Stoke Fruit Farm, Battisford, Suffolk) and around hop bines (B) (Figure 4.3 & 4.4) or stakes (S) which were driven into the ground in close proximity to the hop trellis (at HRI-East Mallings). The bands were distributed in the



Figure 4.3 Trellis in hop garden at HRI, East Malling in winter. Velour and hessian overwintering bands can be seen



Figure 4.4 Velour overwintering band around hop bine

field during October 1998, in areas where *N. californicus* had been found during the summer. They were collected from the apple orchard in February 1999, and from the hop garden on three separate occasions during the winter (January, February and March 1999). The bands were placed in Tullgren funnels (Burkard Agronomics) (one band per funnel), with water in the collecting vessel beneath. Light bulbs (25W) provide a light and heat source which drive any arthropods sheltering within the material placed in the funnel downwards, and into a collecting vessel. Every day the water was filtered and the filter paper examined under a compound microscope. Any arthropods were noted and mites were mounted on slides in PVA mountant, cleared on a hot plate (49°C for 2h), examined using a stereo microscope and identified. The water was examined every day until mites ceased to be found. Once the bands had been removed from the Tullgren funnels, they were scanned under a stereo microscope to check no mites were remaining within. Some samples of leaf litter and hop cones were also taken from the hop garden and the same procedure was followed with the Tullgren funnels.

4.4.1.2 Results

4.4.1.2.1 Hop

Table 4.5 shows the mean number of mites per band obtained. The phytoseiids are split by block and band position as these factors were found to have a significant effect on number obtained per band (see 4.4.2.2.2.1). Table 4.6 shows the mean number of mites obtained per sample for leaf litter and hop cones. Most of the mites sheltering within the over wintering bands, leaf litter or hop cones were obtained after a period of three days in the Tullgren funnels.

Table 4.5 Mean number of mites per band (\pm s.e.) obtained from a hop garden (HRI, East Malling) over a period of extraction in Tullgren funnels.

		Number of days in Tullgren funnels					
		1	2	3	4	5	7
sample size (total no. of bands)		12	12	12	6	6	6
Mean no. of mites per band	phytoseiids (block A3+bine)	1.83 \pm 1.14	0.25 \pm 0.43	0.17 \pm 0.37	0	0	0.17 \pm 0.37
	phytoseiids (block A3+stake)	0.67 \pm 0.85	0	0	0	0	0
	phytoseiids (block 'other'+bine)	1.25 \pm 0.43	0.5 \pm 0.5	0	0	0	0
	tyroglyphids	2.0 \pm 4.25	0	0.12 \pm 0.32	0	0	0.17 \pm 0.55
	tarsonemids	0.1 \pm 0.30	0.1 \pm 0.30	0	0	0	0.08 \pm 0.27
	oribatids	0.03 \pm 0.18	0	0.06 \pm 0.26	0	0	0
	tydeids	0.41 \pm 0.72	0.21 \pm 0.40	0.06 \pm 0.23	0	0	0

Table 4.6 Mean number of mites (\pm s.e.) obtained from leaf litter (LL) and cones (C) from a hop garden (HRI, East Malling) over a period of extraction in Tullgren funnels.

		Number of days in Tullgren funnels			
		1	2	3	4
sample size LL		4	4	4	4
sample size C		3	3	3	3
Mean no. of mites per sample	phytoseiids LL	0.75 \pm 0.82	5.50 \pm 5.89	1.50 \pm 2.59	0
	phytoseiids C	7 \pm 4.55	1.33 \pm 0.47	0	0
	oribatids LL	3.25 \pm 2.38	24.75 \pm 23.95	30.75 \pm 25.68	11.50 \pm 8.38
	oribatids C	9.67 \pm 5.24	14.33 \pm 13.27	8.67 \pm 6.18	1.33 \pm 0.94
	tyroglyphids LL	0.75 \pm 0.83	2.0 \pm 2.35	26.50 \pm 43.60	0
	tyroglyphids C	4.67 \pm 5.90	0	0	0
	tydeids LL	8.25 \pm 3.03	14.25 \pm 12.87	1.25 \pm 1.09	0
	tydeids C	19.33 \pm 11.67	4.67 \pm 2.87	0.67 \pm 0.94	0.33 \pm 0.47

4.4.1.2.2 Apple

For oribatids, the results are grouped into position on tree (trunk vs. branch) and for tydeids the results are grouped into band type (velour vs. hessian) as these factors were found to have a significant effect on number obtained per band (see 4.4.2.2.2.2). Table 4.7 shows the mean number of mites per band obtained. Most of the mites were extracted after four days, although some (tydeids, tyroglyphids and oribatids) were still being obtained after seven days.

Table 4.7 Mean number of mites per band obtained from an apple orchard (Stoke Fruit Fm, Suffolk) over a period of extraction in Tullgren funnels.

		Number of days in Tullgren funnels			
		1	2	4	7
Mean no. of mites per band	phytoseiids	2.50	0.42	0.17	0.08
	tyroglyphids	1.38	0.63	0.17	0.75
	tarsonemids	0.29	0.04	0	0.08
	<i>Zetzellia mali</i> (Ewing)	0.21	0.33	---	0.11
	oribatids - branch	1.08	0.33	0.17	0.17
	oribatids - trunk	8.42	2.83	0.67	1.83
	tydeids – hessian	4.42	1.17	2.00	0.17
	tydeids – velour	299.67	165.42	8.17	170.17

4.4.2 Overwintering of *N. californicus* in apple, hop and blackcurrant plantations

4.4.2.1 Materials and methods

4.4.2.1.1 Hop

A variety of overwintering refuges were placed in the hop garden (HRI plot number: DR149) in October 1998. Bands were placed in the rows highlighted in red, in areas in which *N. californicus* had been found previously (Appendix III, Figure II.1). These were hessian and velour bands, either placed around the hop vines or around stakes which were driven into the ground close to the supporting trellis. In addition, some netting was placed over patches of leaf litter and held in place with large metal staples to see if there were any differences in phytoseiids caught in leaf litter that had been trapped below the trellis as opposed to that which had been blown around. The hop garden was used during the summer for an experiment investigating various methods of aphid and mite control. Some predatory mites

(*N. californicus* and *P. persimilis*) had been released and the refuges were placed in the rows where the releases took place, and other areas in the garden where phytoseiids had been found towards the end of the summer. A random selection of bands and leaf litter were collected on three separate occasions in January 1999, then once in February and once in March. Any arthropods sheltering in them were removed by placing the bands in Tullgren funnels. The mites obtained were mounted on slides in PVA mountant, cleared on a hot plate (49°C for 2h), examined using a stereo microscope and identified. Once the bands had been removed from the Tullgren funnels, they were scanned under a stereo microscope to check no mites were remaining within. After all the phytoseiids had been extracted the bands were measured. A total of 58 bands were collected, 16 of each type (hessian and velour) from the bines and 13 of each type from stakes. During the summers of both 1998 and 1999, there was no effect of row on number of phytoseiids found (Campbell – pers. comm.) therefore it was unnecessary to include this factor in a statistical analysis. The results obtained were analysed for an effect of band material (hessian (H) or velour (V)), band position (bine (B) or stake (S)), hop type (Herald or First Gold) or treatment (A1, A3, C2, 1930C – see Appendix VI) on the numbers of mites obtained per band. The data were found to be normally distributed and the means were tested by two-way ANOVA without replication, except one set of data (mentioned in the results) which was analysed by two-way ANOVA.

In September 1999, hessian and velour bands were placed in 7 rows in an area of the hop garden in which large numbers of phytoseiids had been found previously (the aphid-resistant variety, see Appendix III, Figure II.2). There were no bands placed round stakes as the previous year's results showed that phytoseiids overwintered in the bands secured around the bines, rather than those round stakes. Two dataloggers (TinyTag Plus, Gemini Data Loggers: Temperature range H (-30 → +50°C) & range G Standard probe (-40 → +125°C)), with the external probe located within either a hessian or velour band, were placed in row 20. These monitored the temperature both in the hop garden and within the material bands. A selection of bands chosen at random and hop cones from the hop hedge and leaf litter were collected approximately once a fortnight. Leaf litter itself was not collected as there were no hop-dwelling phytoseiids found in the leaf litter in the previous year, only in the cones that had fallen off the hedge. The bands, and samples of leaf litter and cones, were placed in Tullgren



Figure 4.5 Hop cones on trellis in winter

funnels as before. In total 132 bands (67 hessian and 65 velour), 43 samples of cones (10 cones in each) from the hedge (Figure 4.5) and 49 samples of cones from the leaf litter were collected. All the overwintering bands placed in the hop garden in 1999-2000 were in the same hop variety (an aphid resistant variety) and had received the same treatment. The results obtained were analysed by two-way ANOVA for an effect of band type and row number on mean numbers obtained per band.

4.4.2.1.2 Apple

Plot CW115, HRI-East Malling, Kent, 1998-1999.

At the end of January 1999, 11 overwintering bands that had been placed in an apple orchard over a year previously by a colleague (autumn 1997), were collected and placed in Tullgren funnels as before.

Stoke Fruit Farm, East Anglia, 1998-1999.

A leaf and leaf litter sample were collected from the apple trees in October 1998, and at the same time hessian (H) and velour (V) overwintering bands were placed around the trees. Bands were placed in three rows (A, B or C – see Appendix IV, Figure III.1), some around the branches (B) and some the trunks (T) of the trees. Five bands of each type (HB, HT, VB and VT) were placed in each row, therefore there were 20 bands per row and 60 bands in total. The overwintering bands were collected in February 1999 and placed in Tullgren funnels as before. The results obtained were analysed for an effect of row in which the bands were placed in the orchard and band type (material + position, i.e. HB, HT, VB or VT). This was tested by two-way ANOVA, on the mean numbers per band for each of these factors and for each mite type. A two-way ANOVA was then carried out on the individual replicates (number per band) for an effect of band material type (H or V) or position on tree (T or B) on means.

4.4.2.1.3 Blackcurrant

Upper Horton Farm, Chartham Hatch, Kent, 1998-1999.

Overwintering bands were placed in this blackcurrant plot because *N. californicus* had been found on leaf samples during the summer. Half the plot consisted of pruned blackcurrants that were reduced to approximately a foot high. The rest of the plot was of bushes that had not been pruned and had been in the ground for eight years. At the beginning of November

1998, hessian and velour overwintering bands were placed in four rows, two rows of 'old' blackcurrants and two of the 'young' ones (see Appendix IV, Figure III.2). Bands were placed around the blackcurrant stems or around stakes which were driven into the ground close to the bushes. Some leaf litter was pinned down with netting, and secured by large metal staples so that it would be possible to examine the mite fauna of the leaf litter (the rows in this blackcurrant plantation were kept fairly bare and thus leaf litter blows away during the winter). The bands and leaf litter were collected at the end of January 1999, and placed in Tullgren funnels as before. Unfortunately, during commercial pruning of the plants, many bands were lost and only 50 bands (15 hessian and 15 velour attached to branches, 10 hessian and 10 velour attached to the main stem) remained to be collected plus five samples of leaf litter that had been pinned down. The numbers of mites obtained were too small to be able to carry out statistical comparisons of any effect of band type, plot treatment or band size on the number of mites obtained.

Rosemary Lane, Ticehurst, Kent, 1999-2000.

In September 1999, overwintering bands (hessian and velour) were placed in a young (2 year-old) blackcurrant plantation at Rosemary lane, Ticehurst. There were two varieties of blackcurrants; Ben Gairn and Ben Hope (see Appendix V). Half of each of these varieties had been subjected to a conventional spray programme (fungicide applications (mancozeb/Karamate, chlorothalonil/Bravo, myclobutanil/Systhane and sulphur) and a pyrethroid acaricide (fenpropathrin/Meothrin)) and half to an 'IPM' programme (just the fungicides). A leaf sample taken in August showed there to be more phytoseiids on leaves from the conventional rows than on the 'IPM' ones and this was true for both varieties. Thus, overwintering bands were placed in the conventional rows. Two dataloggers (TinyTag Plus, Gemini Data Loggers: Temperature range H (-30 → +50°C) & range G Standard probe (-40 → +125°C)), the external probe located within either a hessian or velour band, were placed in the Ben Gairn conventional area (Figure 4.6). These monitored the temperature both in the blackcurrant plantation and within the material bands. Approximately six velour and six hessian bands from each variety and some leaf litter were collected in October, November, January and April. The bands and leaf litter were placed in Tullgren funnels as before. Two-way ANOVA with replication was carried out to determine whether variety (Ben Gairn or Ben Hope) and band type (hessian or velour) had an effect on the numbers of phytoseiid mites found sheltering within.



Figure 4.6 Hessian overwintering band around blackcurrant stem with the external probe of a temperature logger (TinyTag Plus, Gemini Data Loggers) within the band

4.4.2.2 Results

4.4.2.2.1 What mites were found overwintering?

4.4.2.2.1.1 Hop

HRI-East Malling, Kent, 1998-99

No *N. californicus* were found; the predominant phytoseiid species was *A. cucumeris* and *T. pyri* was also present. The other mites that were found are shown in Table 4.8. Some of the phytoseiids obtained from the overwintering bands had dark brown guts indicating that they had fed. A small number of *A. cucumeris* and *T. pyri* were found in the hop cones remaining on the trellis in January, or in hop cones that had fallen off the trellis and were lying on the ground. There were no plant-inhabiting phytoseiids found in the leaf litter, therefore there were no differences in the numbers of phytoseiids found in the leaf litter that had been pinned down and that which had not. The 'other' phytoseiid species recorded in Table 4.8 are soil-dwelling ones, and there were greater numbers of these found in the leaf litter that had been pinned down. Figure 4.7 shows the mean number of mites obtained per band throughout the winter.

Table 4.8 Mites found in overwintering bands, leaf litter and cones in a hop garden (HRI, East Malling), 1998-1999.

		mean no. per band				mean no. per sample		
		attached to bine		attached to stake		cones*		
		hessian	velour	hessian	velour	leaf litter	ground	trellis [#]
phytoseiids	<i>A. cucumeris</i>	1.44	2.31	0.15	0.31	0	3.5	1.0
	<i>T. pyri</i>	0.25	0.75	0	0.24	0	0.5	2.0
	<i>Macroseius</i>	0	0.13	0	0	0	0.5	0
	sp.							
	<i>Amblyseius</i>	0	0.06	0.15	0.24	6.75	4.0	0
	sp.							
	other	0	0	0	0	1.25	1.0	0
tydeids		0.75	2.81	1.54	1.23	47.5	33.5	8.0
oribatids		0.13	0.06	0.46	0.31	70.25	48.5	0
tyroglyphids		0.44	0.25	4.77	4.0	29.25	6.5	1.0
tarsonemids		0.19	0.31	0	0.08	0	0	0

* a sample consisted of ten entire cones

[#] only one sample was taken in January, as there were no cones remaining on the trellis at subsequent sample dates

Figure 4.7 Mean number of mites obtained per band throughout the winter.
Hop garden, HRI-East Malling, 1998-1999.

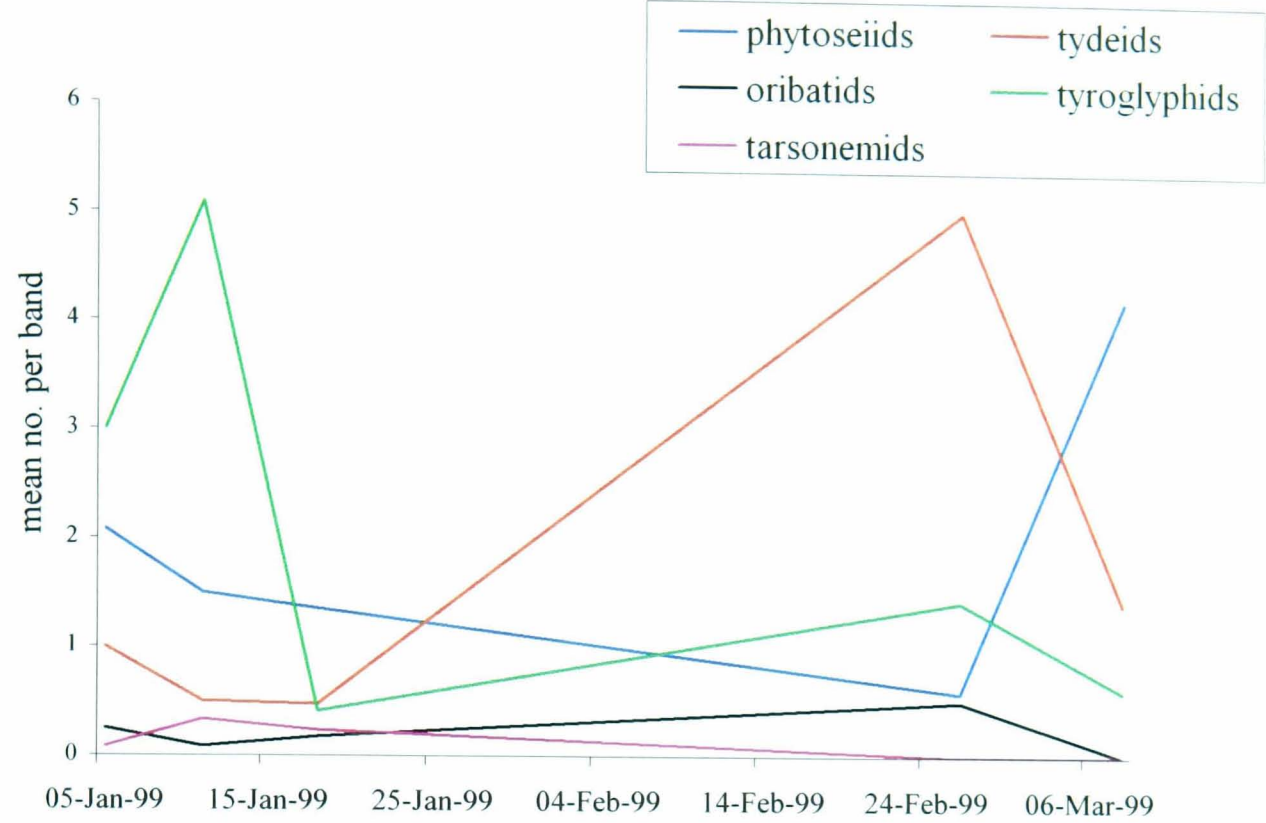
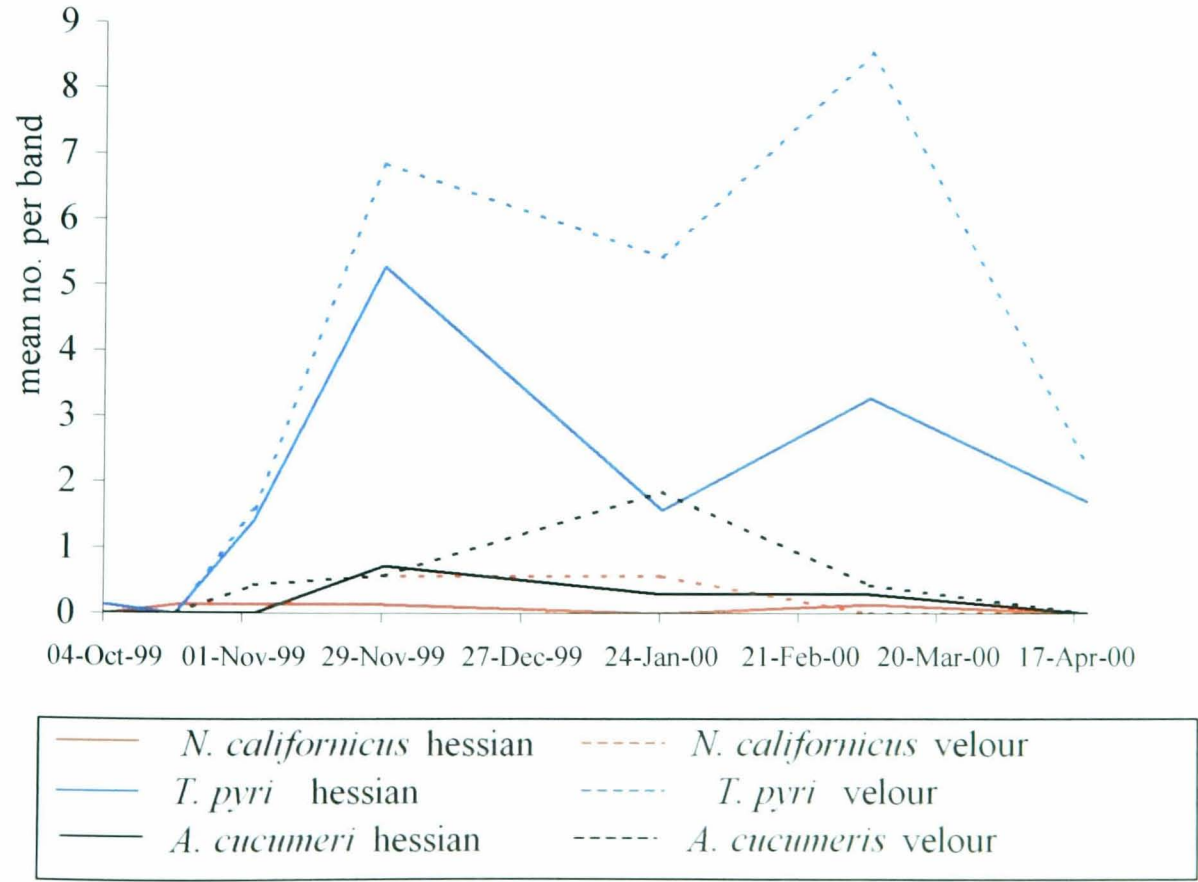


Figure 4.8 Mean number of phytoseiid mites obtained per band throughout the winter. Hop garden, HRI-East Malling, 1999-2000



HRI-East Malling, Kent, 1999-2000

A few *N. californicus* were obtained; a total of 28 individuals from 132 bands and six samples of ten cones from the hop hedge and six samples of cones from the leaf litter. The predominant phytoseiid species found was *T. pyri*, and some *A. cucumeris* were also present. Small numbers of all three of these phytoseiid species were found in hop cones on the trellis, and in hop cones that had fallen off the trellis and were lying on the ground amongst the leaf litter. There were also some soil dwelling phytoseiids found within the cones lying on the ground. A few *P. persimilis* were found in samples of hop cones from the hedge on the first sample date at the beginning of October, however they had disappeared by the next sampling date two weeks later. A few overwintering *T. urticae* were obtained from the bands (6 individuals in total), and from the cones on the hedge (9 individuals). Table 4.9 summarises the numbers and type of mites found overwintering in the material bands and hope cones. Figure 4.8 shows the mean number of phytoseiid mites obtained per band throughout the winter.

Table 4.9 Mites found in overwintering bands, leaf litter and cones in a hop garden (HRI, East Malling), 1999-2000.

		mean no. per band (all attached to bine)		cones - mean no. per sample (10 cones)	
		hessian	velour	ground	trellis
phytoseiids	<i>N. californicus</i>	0.06	0.15	0.06	0.26
	<i>T. pyri</i>	1.40	2.66	0	0.28
	<i>A. cucumeris</i>	0.13	0.35	0.10	0.05
tydeids		0.01	0.12	0	0.20
oribatids		0.03	0.12	3.76	0.69
tyroglyphids		0.01	0	*	0.05
tarsonemids		0.18	1.14	0.47	0.03

*not recorded. Although many were found in the cones from the leaf litter, they were soil-dwelling mites and therefore not included in this survey.

4.4.2.2.1.2 Apple

Plot CW115, HRI-East Mallong, Kent, 1998-1999.

No *N. californicus* were found. The other mites that were found are listed in Table 4.10. Nine spiders and one earwig were also found sheltering within the bands.

Table 4.10 Mean no. of mites obtained per band from an apple orchard (HRI-East Mallong), January 1999.

		mean no. of mites per band	
		hessian	velour
phytoseiids	<i>T. pyri</i>	0.16	2.60
	<i>A. rhenanus</i>	1.33	4.60
	<i>E. finlandicus</i>	0	0.20
	<i>Macroseinae</i> sp.	0.50	7.8
oribatids		7.17	20.8
tydeids		0.83	53.4
tarsonemids		0	0.60
tyroglyphids		15.67	12.80

Stoke Fruit Farm, East Anglia, 1998-1999.

Only a few *N. californicus* were found (two individuals in a total of 60 bands). All the mites found are listed in Table 4.11. The predominant phytoseiid species found was *T. pyri*, and some *A. cucumeris* and *Anthoseius rhenanus* (Ouedemans) were also present. A staggering number of tydeids (>200 per band) were found in the velour bands.

Table 4.11 Mean no. of mites per band from an apple orchard, Stoke Fruit Farm, East Anglia, 1998-1999.

		mean no. of mites per band			
		trunk		branch	
		hessian	velour	hessian	velour
phytoseiids	<i>N. californicus</i>	0	0	0.06	0.06
	<i>T. pyri</i>	2.20	1.00	0.80	4.80
	<i>A. cucumeris</i>	0	0.06	0	0
	<i>A. rhenanus</i>	0.06	0.40	0	0.33
	<i>Macroseiinae</i> sp.	0.06	0.13	0	0
<i>Zetzellia mali</i>		1.07	1.87	0.47	1.93
oribatids		16.07	26.87	3.27	6.53
tydeids		3.40	325.53	6.07	219.80
tarsonemids		0.20	0.93	0.21	0.40
tyroglyphids		4.80	4.20	1.67	1.60

4.4.2.2.1.3 Blackcurrant

Upper Horton Farm, Chartham Hatch, Kent, 1998-1999.

No *N. californicus* were found overwintering in the bands. Only 14 phytoseiids were obtained from 50 bands, and of these the predominant phytoseiid species was *T. pyri*. Seven tydeids, 14 oribatids, one tyroglyphid and one tarsonemid were also recorded. A summary of the mites obtained can be found in Table 4.12. No phytoseiid species that were present during the summer on the blackcurrant plants were found in the leaf litter. The only mites present in the leaf litter were soil-dwelling phytoseiids and large numbers of tydeids (>85 in five samples) and oribatids (>435 in five samples).

Rosemary Lane, Ticehurst, Kent, 1999-2000.

Only one *N. californicus* was found from 87 bands. The predominant phytoseiid species found overwintering in the bands was *T. pyri*. In addition a few *A. cucumeris* and another *Amblyseius* species were found. Some of these *Amblyseius* species were also recovered from the leaf litter samples, however none of the other phytoseiids were. A summary of the mites obtained can be found in Table 4.13. Figure 4.9 shows the mean number of mites obtained per band throughout the winter.

Table 4.12 Mites found in overwintering bands and leaf litter in a blackcurrant plantation (Upper Horton, Chartham Hatch), 1998-1999.

	mean no. per band				mean no. per
	attached to stem		attached to stake		sample
	hessian	velour	hessian	velour	leaf litter
<i>T. pyri</i>	0.13	0.20	0.30	0.10	0
<i>Amblyseius</i> sp.	0.13	0.27	0	0	0
tydeids	0.07	0.07	0.10	0.40	17.0
oribatids	0.33	0.27	0.40	0.10	87.0
tyroglyphids	0	0	0.10	0	---*
tarsonemids	0	0	0	0.10	---

* not recorded

Table 4.13 Mites found in overwintering bands and leaf litter in a blackcurrant plantation (Rosemary Lane, Ticehurst), 1999-2000.

		mean no. per band				mean no. per sample
		Ben Gairn		Ben Hope		
		hessian	velour	hessian	velour	leaf litter
phytoseiids	<i>N. californicus</i>	0	0	0.05	0	0
	<i>T. pyri</i>	0	0.09	0.14	0.18	0
	<i>A. cucumeris</i>	0	0.09	0	0.05	0
	<i>E. finlandicus</i>	0	0	0	0	0
	<i>Amblyseius</i> sp.	0	1.27	0	0.14	0.09
	tydeids	0.14	0.18	0.14	0.27	---*
	oribatids	0	0.05	0.05	0	---
	tyroglyphids	0	0.14	0	0.05	---
	tarsonemids	0	0.18	0.05	0.09	---

* not recorded

Figure 4.9 Mean number of mites obtained per band throughout the winter. Blackcurrant plantation, Rosemary Lane, Ticehurst, 1999-2000.

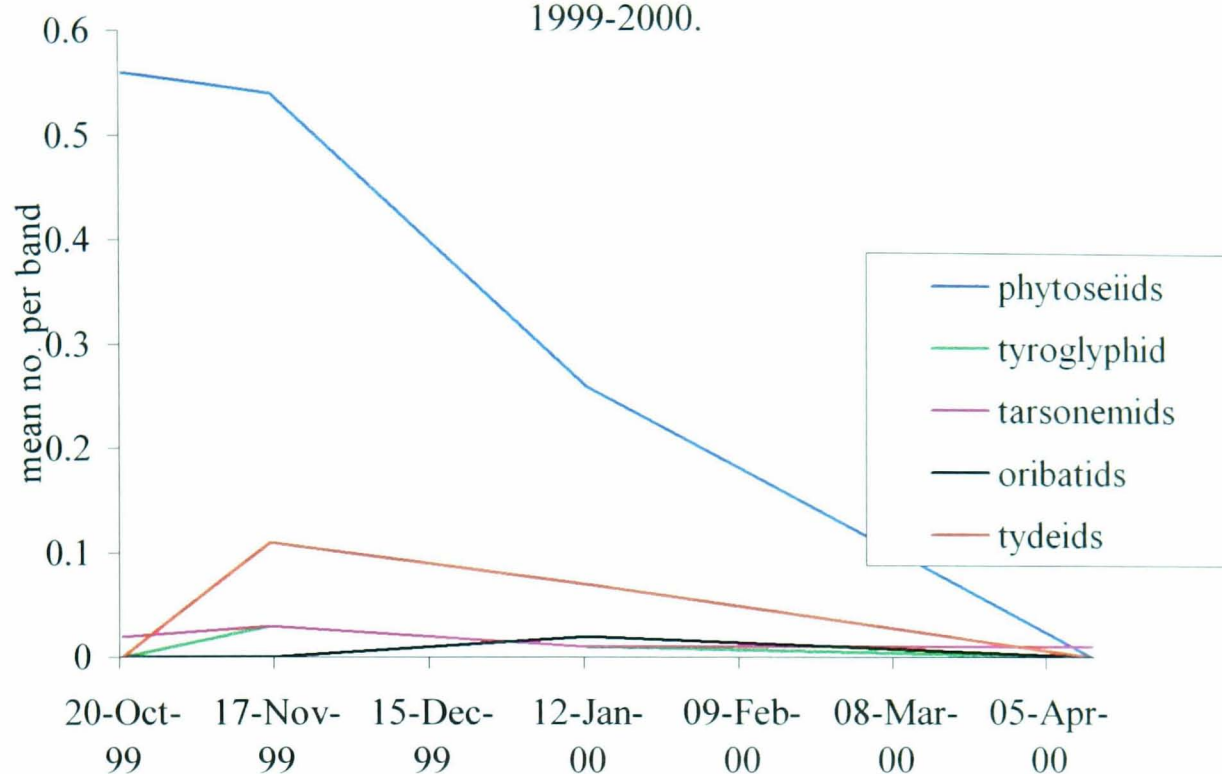
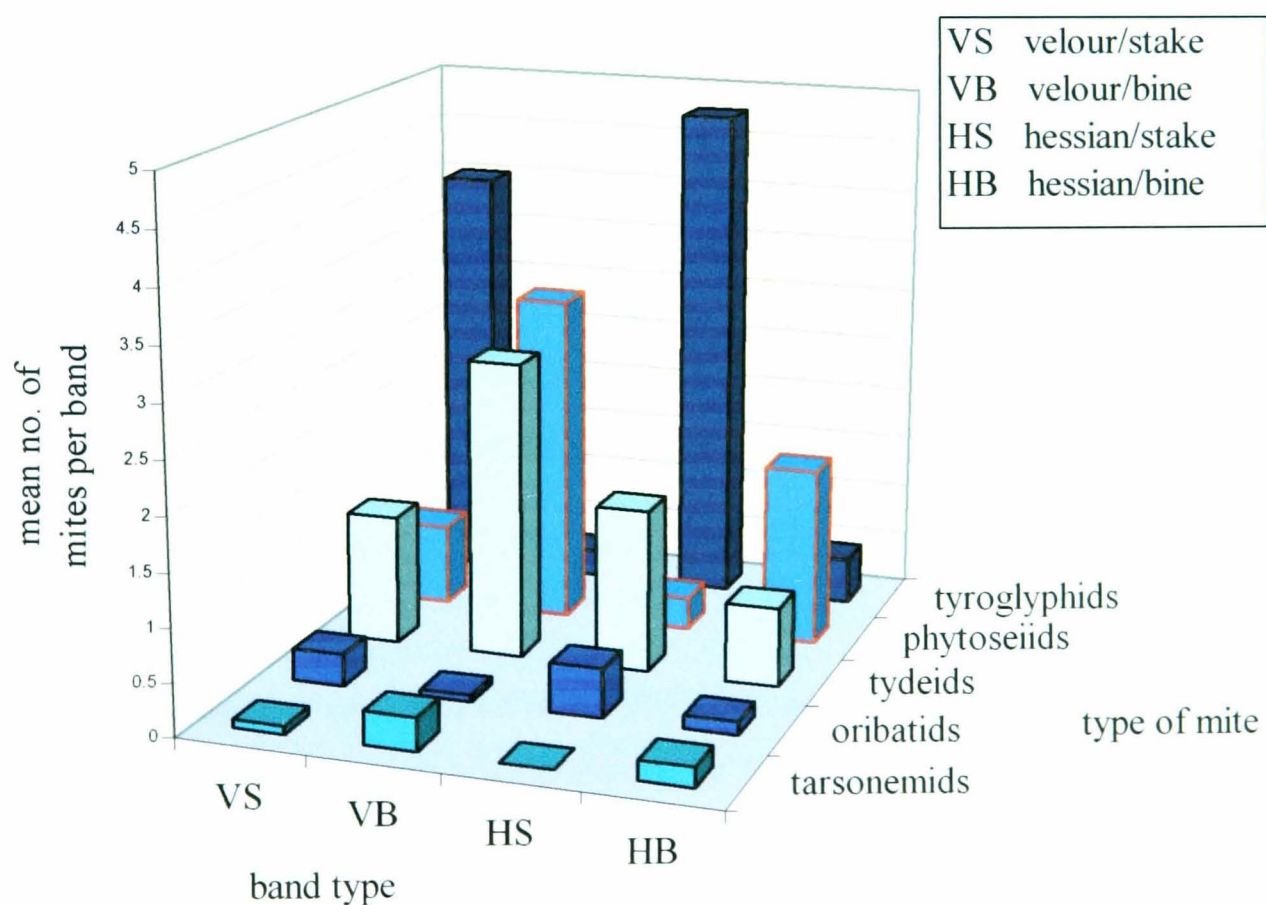


Figure 4.10 The effect of refuge design on abundance and type of mites recovered from a hop garden (HRI, East Malling, 1998-99)



4.4.2.2.2 Is there any effect of band type, variety or plot on the numbers of mites overwintering within?

4.4.2.2.2.1 Hop

East Malling, 1998-99.

For phytoseiids there was found to be a significant effect of both band type (material + position, i.e. HB, HS, VB, VS) and block (A3 or 'other') ($p < 0.05$), for the other mites there was no significant differences in means found. Figure 4.10 is a bar chart showing the effect of refuge design on the numbers of different types of mites recovered. There was no significant effect of material type (hessian or velour), however there was a strongly significant effect of position ($p < 0.01$) with the greater numbers of phytoseiids found in the bands attached to the hop bines. Greater numbers of tyroglyphids were obtained from bands attached to stakes, whereas greater numbers of phytoseiids and tarsonemids were obtained from bands attached to hop bines. Tydeids were recovered from all band types but showed a preference for velour bands attached to hop bines.

There was no significant effect of plot treatment (A1, A3, C2 or 1930C - see Appendix VI) on the number of phytoseiid mites overwintering. The band position (i.e. bine or stake) was used as a comparison and this was still significant ($p < 0.05$).

There was no significant effect of either variety (First Gold or Herald) or treatment+position (A3+bine, A3+stake, C3+bine, C3+stake) on mite numbers. An equal number of replicates were available for the A3 treatment. Thus, a two-way ANOVA with replication was carried out on this treatment comparing position (bine or stake) with variety. Again, there was no significant effect of variety on the numbers of phytoseiids obtained, and band position was still significant ($p < 0.01$).

East Malling, 1999-2000.

In 1998-99 there was no effect of row number on phytoseiids found. However, the 1999-2000 study was conducted on a different part of the hop garden, and when the results were analysed there was found to be a significant effect of both band type and row number ($p < 0.05$), with the greater number of phytoseiid mites being obtained from velour bands. The row numbers, listed in order with the average number of mites per row going from least to most, were: 9, 10, 16, 20/15, 6, 2.

4.4.2.2.2 *Apple*

Stoke Fruit Farm, East Anglia, 1998-1999.

In a comparison of row in which the bands were placed in the orchard and band type (material + position, i.e. HB, HT, VB or VT) there was found to be no significant differences in mean numbers of mites obtained per band for most types of mite. The exception was an effect of band type on oribatids ($p < 0.005$). An examination of the effect of band material type (H or V) or position on tree (T or B) on means showed that there was no significant differences in means except for an effect of position on oribatids with larger numbers occurring on the trunk ($p < 0.05$). In addition, for tydeids there was a significant difference in means for material type with larger numbers occurring in velour bands ($p < 0.05$).

4.4.2.2.3 *Blackcurrant*

Rosemary Lane, Ticehurst, Kent, 1999-2000.

There was no significant effect of variety on numbers of phytoseiids, however there was a significant effect of material type ($p < 0.05$) with more phytoseiids being recovered from velour bands.

Table 4.15 summarises the effect of band type, variety or plot treatment on numbers of mites obtained from the various sites where overwintering bands were put out.

Table 4.15 The effect of band type, plot treatment, variety or row number on the quantity of phytoseiids found in overwintering refuges. A summary of the results.

Host plant	location	was there an effect of this variable on phytoseiid numbers?			
		band type	plot treatment	variety	row no.
hop	East Malling, 98-99	yes (preference for bands on bines)	no	no	no
hop	East Malling, 99-00	yes (preference for velour)	no	---	yes
apple	Stoke Fruit Farm, 98-99	no	no	no	no
blackcurrant	Ticehurst, 99-00	yes (preference for velour)	no	no	no

4.4.2.2.3 *Is there an effect of band size on numbers of mites overwintering within?*

Figures 4.11 and 4.12 are scatter diagrams, on which are plotted the number of mites obtained against band area for the bands collected from hop and apple. For the bands collected from hop in 1998-99, a distinction is made between the bands attached to the bines (blue) and those attached to stakes (red). For those collected in 1999-2000, a distinction is made between band material, as this was shown to have a significant effect on mite numbers.

The points on the graphs are widely scattered, showing that there is no correlation between band area and numbers of phytoseiids found within.

4.4.2.2.4 *Were any other beneficial arthropods found sheltering in the overwintering bands?*

The other beneficial arthropods found sheltering in the overwintering refuges are summarised in Table 4.16. There were also many earwigs, coccinellids and centipedes found sheltering in between the bands and the trunk of the trees in the apple orchard at Stoke Fruit Farm in 1999-2000.

Figure 4.11 Numbers of phytoseiids obtained from overwintering bands of varying size. Hop garden, HRI, East Malling.

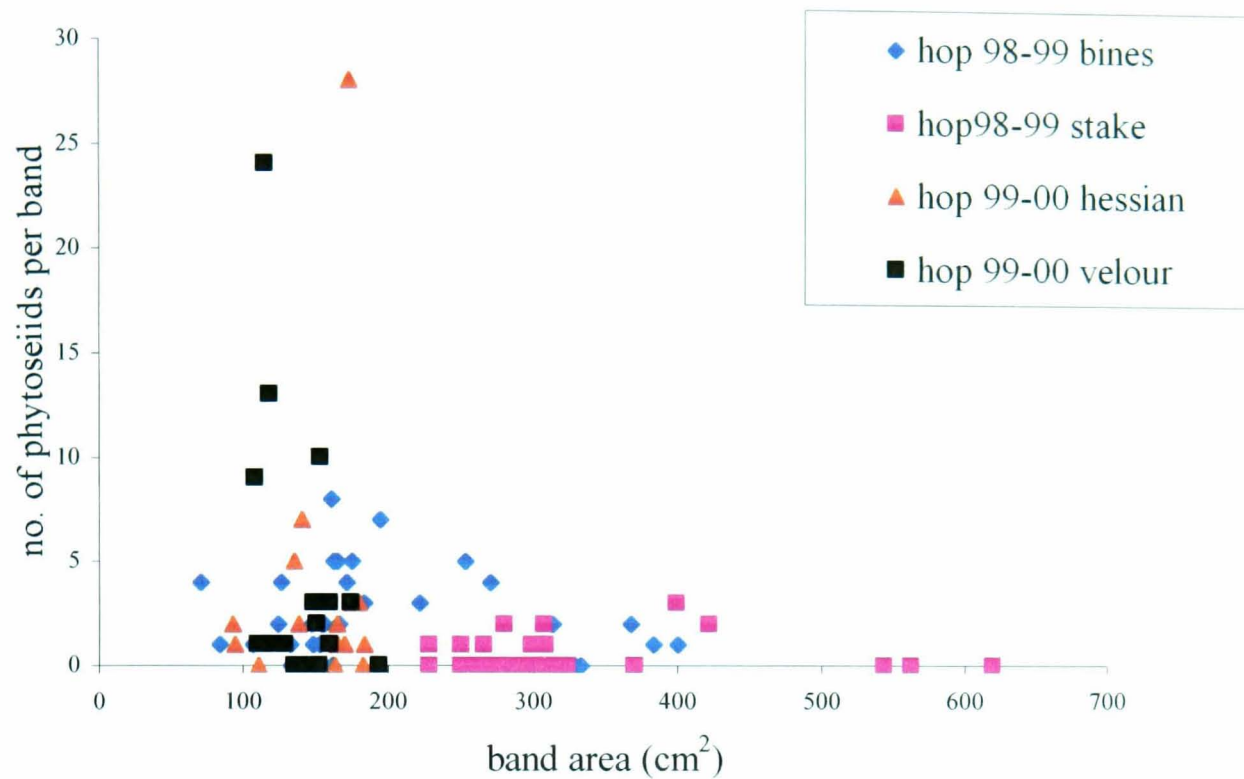


Figure 4.12 Numbers of phytoseiids obtained from overwintering bands of varying size. Apple orchard, Stoke Fruit Farm, East Anglia, 1998-99.

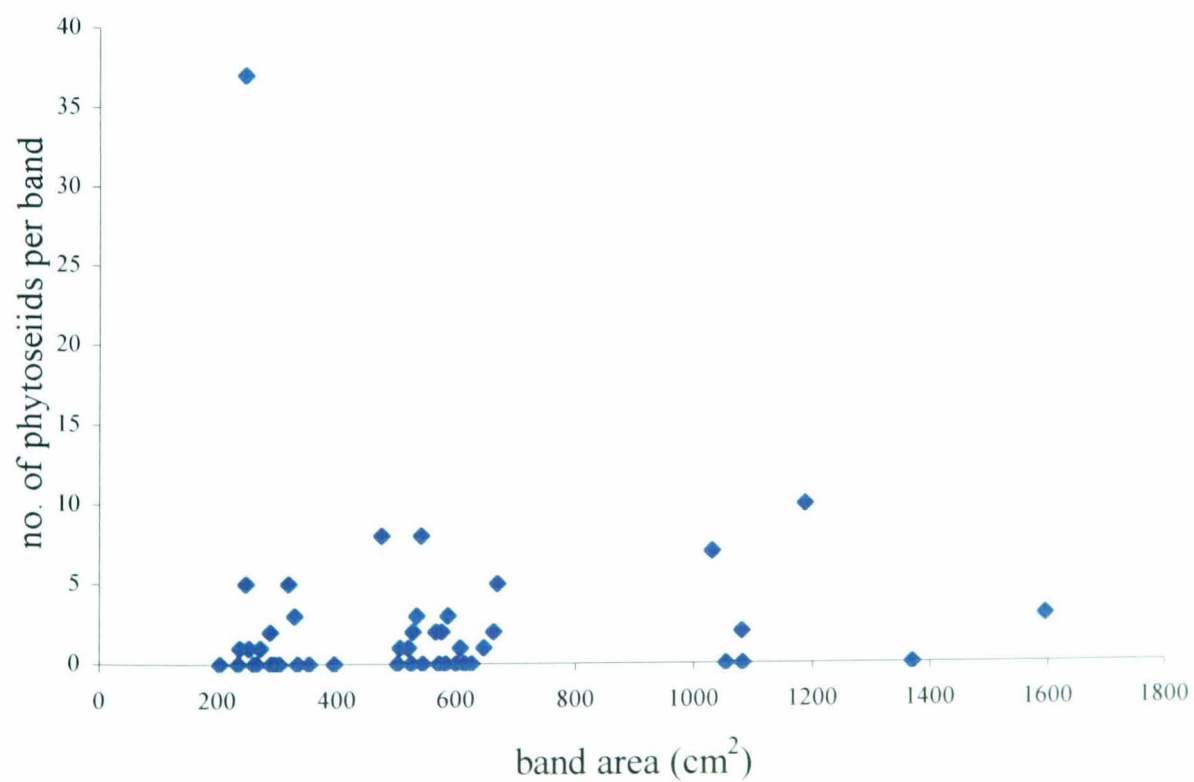


Table 4.16 A summary of the other beneficial arthropods found overwintering in refuges in hop, apple and blackcurrant plantations.

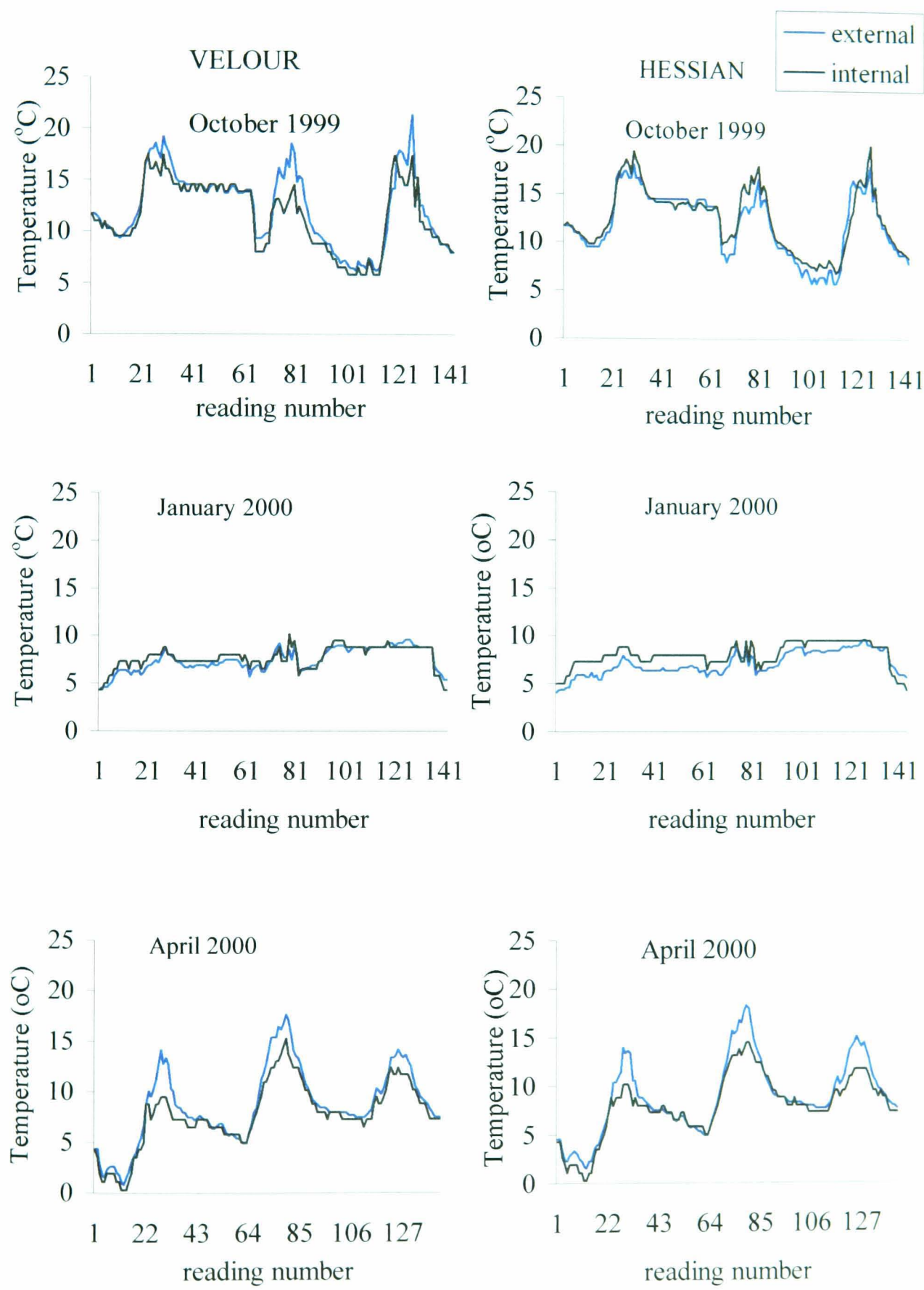
host plant	hop	hop	apple	blackcurrant	blackcurrant
Location	EM, 98-99	EM, 99-00	Stoke Fruit Fm, 98-99	Upper Horton, 98-99	Ticehurst, 99-00
Total no. of bands	58	132 cones (43 samples)	60	50	87
<i>Chrysoperla</i> <i>carnea</i> (Stephens) adults (lacewings)	2	1	0	0	0
syrphid larvae (hoverflies)	1	1	0	1	0
anthocorrids	6	1	5	0.12/band	3
mirids	2	1	0	0	0
arachnids (spiders)	0	6	1	1.22/band	16
forficulids (earwigs)	0	0	0	0.62/band	0
staphylinid beetle larvae	0	0	0	1	1

4.4.2.2.5 What was the internal and external temperature difference in the overwintering bands?

4.4.2.2.5.1 Hop

The mean internal temperature of the velour band was 6.75°C, whereas the mean external temperature recorded by the same logger was 7.04°C. The mean internal temperature of the hessian band was 6.72°C, and the mean external temperature 6.71°C. Figure 4.13 shows graphs of the temperatures recorded over several days in autumn, winter and spring (October, January & April) from a hessian and a velour band in the hop plot, HRI-East Malling, and shows a comparison of the temperature fluctuations within the bands compared to ambient ones.

Figure 4.13 Temperatures recorded over three days inside & outside an overwintering band. Readings were taken every 30 mins.
Hop, HRI, East Malling, 1999-00.



4.4.2.2.5.2 *Blackcurrant*

The mean internal temperature of the velour band was 6.67°C. Unfortunately the channel of the logger that recorded ambient temperature failed to work. The mean internal temperature of the hessian band was 6.55°C, and the mean external temperature was 7.26°C.

4.4.3 Discussion

Most of the mites sheltering within overwintering bands were obtained after a period of four days in the Tullgren funnels. Whereas for samples of leaf litter or hop cones all the phytoseiids had been extracted after three days, presumably because these dry out quicker and afford less protection than material bands. For the mites which seem to decrease in number over a period of extraction and then start to increase again (e.g. tyroglyphids and tarsonemids from hop (Table 4.5) and tydeids from velour bands on apple (Table 4.7)) it is possible that some of these overwinter deep within the material bands and thus emerge after a time delay when placed in the funnels. Therefore, for these mites a longer extraction period of >7 days would be recommended if a total count was required. This did not appear to be the case for phytoseiids and it was concluded that an extraction period of at least four days, and optimally seven, be employed. It should be noted that the standard error values in Tables 4.5 and 4.6 are high because some bands contained no mites at all, and others large numbers; resulting in a large variation.

The predominant and widespread phytoseiid present overwintering in all the locations studied was *T. pyri*. *Amblyseius cucumeris* and *E. finlandicus* were also fairly common. There were few *N. californicus* found overwintering. Of the six sites examined over the three-year period only half had *N. californicus* present. However, in the regions where it was found, it persisted throughout the winter, albeit in low numbers.

No *N. californicus* were recovered from leaf litter. In Uruguay apple orchards the preferred overwintering site was found to be in the bark of the tree trunk or in the buds (Bruhn & Beltrame, 1981). On hop phytoseiids were found in the cones on the hedge, and also in those lying on the ground presumably carried there when the cones fell from the bines. Cones remain on the hedge for some time after the leaves have fallen off and also contained some tydeids, tyroglyphids and overwintering *T. urticae* and therefore may be an attractive overwintering site; providing both shelter and a food source. In southern France, Raworth *et*

al. (1994) found that *N. californicus* was present on apple leaves on trees and in the ground cover before leaf fall. Many phytoseiids were carried to the ground with the leaves when they dropped off the trees and were observed in the dead leaves in autumn. Whilst none were found in apple leaf litter in the present study, it would appear that *N. californicus* are demonstrating this strategy on hops.

From Figures 4.7 and 4.8 it can be deduced that mites enter their overwintering site between the end of October and mid-November, and leave around the beginning of March. When considering overwintering surveying using such refugia, it would therefore be optimal to put the bands in the field by the beginning of October. Similar results were obtained by Raworth *et al.* (1994) in a study of apple trees in southern France. They captured *N. californicus* up to mid-November, then from the end of February found actively searching *N. californicus* in the apple trees and the ground cover. No immatures or males were found until 10 March, which suggested that the start of the first generation was around the middle of March.

The material type did not appear to be very significant, although where a preference was shown velour had greater numbers overwintering within. This, however, is different to the findings of Greatorex (1997) who collected a greater number of phytoseiids from hessian bands. However, her graphs show *T. pyri* to be greater in number in velour bands from two out of three of the plots studied and a distinction was not made between material and those attached to the branch and those to the trunk (which had been found to be significant variables by themselves).

Overwintering band size had no effect on numbers of phytoseiids obtained. This is to be expected, as it is unlikely that competition for space or resources would occur between a few small mites in bands of that size. Low numbers of beneficial arthropods were also found exploiting the refuges, and in this instance they were probably too small to afford shelter to many larger arthropods. Large numbers of earwigs, coccinellids and centipedes were found at Stoke Fruit Farm and the only obvious difference between this orchard and a conventional one was that no organophosphorous insecticides had been used there since 1996. Instead a pyrethroid programme has been followed. It would be interesting if this was the reason as pyrethroids are also broad-spectrum insecticides and would not be expected to benefit these species.

The mean temperature within the bands in the hop garden was higher than the external temperature. However, this was not the case for the blackcurrant plantation. Refuges undoubtedly act as buffers of temperature fluctuation, rather than providing a definitively warmer environment. Rapid temperature changes are most likely to have cold-shock effects on exposed arthropods, causing chilling injuries. If an overwintering site affords buffering from sudden temperature fluctuations it would be advantageous to exploit it.

It should be noted that using overwintering bands as a monitoring technique provides a snapshot of what species is overwintering rather than a total count. It is not known whether a certain species will overwinter preferentially in material bands or in other sites afforded by the host plant, such as cracks and crevices in the bark.

4.5 Overall Discussion

This study has established that *N. californicus* has the ability to diapause, and this ability may be genetically determined and influenced by its geographical origin. Its SCP ranged from approximately -20 to -23°C and pre-freeze mortality occurred. However, survival at brief exposure to -10°C was 100% and survival at longer exposure to -5°C was greater than that of the chill-susceptible species *P. persimilis*. Survival time increased when *N. californicus* had been reared in SD conditions. The response of *N. californicus* to the low temperature experiments indicated that it is a chill tolerant species. The refugia experiments showed that *N. californicus* was able to survive the winter in the UK.

In conclusion, as *N. californicus* has the ability to diapause, is a chill tolerant species, and was found in overwintering refugia throughout the winter in recent years, it can be assumed that it does have the potential to survive winter conditions in the UK and, therefore, to establish.

CHAPTER 5: POTENTIAL AS A BIOCONTROL AGENT

5.1 Introduction

Previous studies on various aspects of the biology of *Neoseiulus californicus*, including development rates and fecundity, have concentrated on certain prey species. These include tetranychid species (Castagnoli & Ligouri, 1991; Castagnoli & Simoni, 1994; Gilstrap & Friese, 1985; McMurtry, 1977; Mesa *et al.*, 1990; de Moraes & McMurtry, 1985; Monetti & Croft, 1997) and some from other mite families: *Oligonychus pratensis* (Banks) (Pickett & Gilstrap, 1986a), *Frankliniella occidentalis* (Pergande) (Rodriguez-Reina *et al.*, 1992) and *Polyphagotarsonemus latus* (Castagnoli & Falchini, 1993; Peña & Osborne, 1996).

McMurtry (1982) suggested six characteristics that may be important when considering how effective a predator may be: powers of dispersal, distribution in relation to prey, reproductive potential, voracity, specificity, and ability to survive when prey is scarce. *Neoseiulus californicus* demonstrates good dispersal ability and has been found on various host plants (Fauvel *et al.*, 1993); it can survive seven days of starvation (Palevsky *et al.*, 1999).

According to McMurtry and Croft's categorisation of phytoseiid life-styles (McMurtry & Croft, 1997), *N. californicus* is a Type II predator. Their proposed life-style categories were: Type I, specialised predators of *Tetranychus* species; Type II, selective predators of tetranychid mites and especially associated with those that produce dense webbing; Type III, generalist predators. McMurtry and Croft made some generalisations about Type II predators; they show less of a response to prey density than *Phytoseiulus* species, but some show a strong aggregation response to leaves infested with *T. urticae*. Type II species can reproduce on mites in other (non-tetranychid) families. *Neoseiulus* species are 'known to feed on' eriophyids, and feeding on Tenuipalpidae in the laboratory has been reported for *N. californicus* (Tanigoshi, 1982). The greater activity of species such as *N. californicus* might contribute to stabilising predator/prey systems when the average prey densities are low. Type II phytoseiids are adapted to the conditions created by modern agriculture, having the ability to disperse and reproduce rapidly, and also a tolerance of changing conditions combined with the ability to adapt quickly (McMurtry & Croft, 1997). Castagnoli and Simoni (1999) found the predominant functional response to be Type II curves for *N. californicus* fed on *Tetranychus urticae*. Previous feeding history was discovered to have an effect on the

functional and numerical response, with a wild strain giving better performance than those fed on pollen or dust mites (*Dermatophagoides farinae* Hughes) in the laboratory. Within-plant dispersal of *N. californicus* was greater than that of *Neoseiulus fallacis* on lima bean (*Phaseolus limensis*) and apple, leading McMurtry and Croft (1997) to conclude that their dispersal traits were more like those of a generalist predator compared with *N. fallacis*. They suggested that *N. californicus* is better adapted to control prey that are less aggregated than *T. urticae*, moving more often between prey patches but showing less turning and local exploration than *N. fallacis*. Croft *et al.* (1998a) suggest that *N. californicus* falls between a specialist Type II species and a generalist Type III. They found the reproductive capability of *N. californicus* to be equal to or greater than that of *N. fallacis* (Type I selective predator) when held with excess *T. urticae*, *Panonychus ulmi*, *Frankliniella occidentalis* or maize pollen, but less with *Aculus schlectendali* Napela or *Oligonychus ilicis* (McGregor).

Neoseiulus californicus was found to be most efficient as a predator at a rate of one tetranychid egg/73.3cm² of bean (*Phaseolus vulgaris*) leaf surface, and adult prey at a rate of 1/35.7cm² (Gilstrap & Fries, 1985). Protonymphs and adults recognised and killed most of the prey eggs they encountered. However, protonymphs killed only half the tetranychid protonymphs they encountered, and adults less than a quarter of the adult tetranychids that they came into contact with (Gilstrap & Fries, 1985). Fries and Gilstrap (1982) found that as prey availability increased, total and daily fecundities, number of prey (*Tetranychus cinnabarinus*) killed per day and per ovipositional period, and sex ratio in favour of females, increased. The number of reproductive days also increased, but at higher levels of prey availability (5-10 prey eggs/day) this trend plateaued. The authors suggested that *N. californicus* has a fixed potential fecundity causing a limit to the increase in ovipositional rate and decrease in ovipositional period. At lower levels of prey availability *N. californicus* showed greater total fecundity, number of reproductive days, daily fecundity and number of prey killed during reproductive period than *Phytoseiulus persimilis* or *Metaseiulus occidentalis*. However, at higher levels of prey availability *P. persimilis* showed a greater response in all categories.

In a comparison of nymphal feeding drive and predation by *N. californicus* and *N. fallacis* it was found that there were low rates of nymphal feeding by both predators, and *N. californicus*

displayed a greater amount of intra- and interspecific predation than *N. fallacis* (Monetti & Croft, 1997). This may suggest that *N. californicus* is more of a generalist than *N. fallacis*. The larvae of *N. californicus* may feed if prey is present; Croft *et al.* (1999) described them as 'facultative feeding larvae'. In a study carried out by Palevsky *et al.* (1999), the incidence of larval feeding was very low. They observed larvae of *N. californicus* held with *T. cinnabarinus* for two minute periods at 6h and 12h old and only 4.0% and 1.3% (respectively) were observed to feed. They found no significant differences between larval survival of those held with or without food, and no cannibalism was observed.

Neoseiulus californicus will feed on phytoseiid eggs; immatures are able to complete development and adult females oviposit when feeding exclusively on phytoseiid eggs of another species (Schausberger & Croft, 1999). Palevsky *et al.* (1999) found that, when held in arenas with four *N. californicus* and four *Typhlodromus athiasae* Porath and Swirski eggs, *N. californicus* consumed 2.8 *T. athiasae* and 0.4 of their own eggs per day. *Neoseiulus californicus* is able to discriminate between its own eggs and those of another species, and demonstrated a preference for heterospecific eggs (Schausberger & Croft, 1999). When phytoseiid eggs were soaked in deionised water for 30 minutes, cannibalism increased (Palevsky *et al.*, 1999), leading to the suggestion that the compounds involved in egg recognition are water-soluble.

It is possible that as prey density increases above a certain threshold, the functional and numerical responses of the predator decrease producing a dome-shaped response curve (Holling, 1961). This may be due to 'interference', for example abandoning prey due to mechanical contact with another prey item, or a decrease in manoeuvrability of the predator as the amount of tetranychid webbing increases (Laing & Osborne, 1974). Laing and Osborne (1974) examined the effect of *T. urticae* density on *N. californicus* and were unable to determine a decline in functional or numerical response with increasing prey density.

5.2 The potential of *N. californicus* as a biocontrol agent: a literature review

5.2.1 *Tetranychus urticae*

Castagnoli and Amato (1991) examined the interaction between *N. californicus* and *T. urticae* in the laboratory. Using an Italian strain of *N. californicus* it was found that the introduction of one fertilised female into a *T. urticae* population of approximately 148 individuals (68% of which were eggs), resulted in a 78% reduction in population density after 13 days. If the number of initial *N. californicus* females was increased to two the *T. urticae* population could be eliminated in 13 days, and four initial females resulted in elimination in eight days. *Neoseiulus californicus* showed a preference for immature stages of *T. urticae* even when they were not the most numerous stages (Castagnoli & Amato, 1991). When *N. californicus* nymphs and adults were presented with all stages of prey, a preference for eggs and larvae of *T. urticae* was shown (Mwambula, 1998).

In a study of *N. californicus* and *T. urticae* on strawberry, Greco *et al.* (1999) showed that *N. californicus* had a lower tendency to aggregate, a significant dispersal capacity and an ability to detect which strawberry leaflets were infested even at low prey densities. These factors led them to suggest *N. californicus* as a promising candidate for controlling *T. urticae* on strawberry. Auger *et al.* (1999) examined the factors affecting dispersal of *N. californicus*, feeding on *T. urticae* on dwarf alfalfa (*Medicago polymorpha*). They found that temperatures of >12.7°C induce ambulatory behaviour and high temperatures (35°C), high light intensities and drought-stressed plants increase dispersal. Food availability and high RH (80%) slowed down dispersal activity. Deutonymphs and young ovipositing *N. californicus* females were the stages most likely to respond to dispersal-inducing conditions (Auger *et al.*, 1999). Rott and Ponsonby (2000) also found high RH to adversely influence the activity and predation of *N. californicus*.

In a comparison of strawberry and tomato, the egg to egg development time for *N. californicus*, feeding on *T. urticae*, was unaffected by host plant (Castagnoli *et al.*, 1999). However, mortality was lower and oviposition greater on strawberry, leading Castagnoli *et al.* (1999) to suggest that strawberry is a more suitable host plant for *N. californicus* than tomato, and that this should be taken into consideration when planning biocontrol programs.

In Spain, naturally occurring populations of *N. californicus* were able to control *T. urticae* on strawberry and keep them below economically damaging levels (Garcia-Mari & Gonzalez-Zamora, 1999). In S. California, *N. californicus* has been used to control *T. urticae* on strawberry successfully (Oatman *et al.*, 1977a & 1977b). *Phytoseiulus persimilis* was more effective than *N. californicus* at controlling *T. urticae*. However, plots treated with *N. californicus* gave almost twice as much yield as those with no predators and only slightly less than those treated with *P. persimilis*. *Neoseiulus californicus* did not readily disperse from the release beds to adjacent ones, whereas *P. persimilis* did (Oatman *et al.*, 1977a). Early (Feb 13) releases at 10 predators/plant (as opposed to 5/plant) gave the most effective control (Oatman *et al.*, 1977b).

Neoseiulus californicus and *P. persimilis* have been used in combination as part of a successful IPM program for the control of *T. urticae* on greenhouse-grown poplar (*Populus* sp.). After a year of management under the IPM program only spot applications of acaricide were necessary, mite populations were suppressed to acceptable levels and management costs reduced by 81% (Smith *et al.*, 1993). Heindrickson (1980) used *N. californicus* and *P. persimilis* in combination to satisfactorily control *T. urticae* on alfalfa (*Medicago sativa*) in the glasshouse. *Phytoseiulus persimilis* and *N. californicus* were found to be an effective combination against *T. urticae* in glasshouses (Fournier *et al.*, 1985).

Phytoseiulus persimilis and *N. californicus* were released in corn (*Zea mays*) fields in Texas to examine their effect on *Oligonychus pratensis* and *T. urticae*. Both predators significantly reduced the population of the spider mites and maintained them below economic thresholds. Plots treated with *P. persimilis* generally had lower densities of spider mites than those treated with *N. californicus*, however these differences were only statistically significant on one occasion (Pickett & Gilstrap, 1986b). Plant phenology may be an important factor in timing releases; spider mite growth rates were at a maximum during the 'tassel' phase of corn development. If phytoseiids are released before tasseling they can establish themselves before the spider mites reach their peak growth rates (Pickett & Gilstrap, 1986b). *Neoseiulus californicus* was released in corn in W. Texas, to investigate its persistence as a predator of *O. pratensis*. It was recovered 31 days after its release, and in greater numbers than the other predatory mites (*P. persimilis* and *T. occidentalis*) (Gilstrap *et al.*, 1977). It was proposed as

a suitable candidate for studies involving augmentation of predators. It is interesting to note that it survived conditions of high temperatures, winds and generally low relative humidity.

Neoseiulus californicus has been shown to colonise cotton (*Gossypium* sp.) infested with tetranychid mites (*Tetranychus pacificus*, *Tetranychus turkestanii* Ugarov & Nikolskii and *T. urticae*). The predator caused a decline in tetranychid density at a level of about 0.5/leaf, and at release rates of a predator:prey ratio of 1:30 adequately suppressed the spider mite population. A release rate of 1:20 resulted in a reduction of tetranychid infestation of 60-64%, and a rate of 1:10 a reduction of 91-96% (Tijerina-Chavez, 1991). It has also been shown to give significant control of the spider mite *Tetranychus kanzawai* Kishida on tea in Taiwan. When an average of 150 predators/plant were released the percentage of infested buds in the plots treated with *N. californicus* was 14% compared with 41% in the control plots (Hsaio, 1988).

Combined releases of *P. persimilis*, *Phytoseiulus longipes* Evans and *N. californicus* were more effective at controlling *T. urticae* than single-species releases on tomato, corn, pepper and green bean. However, single-species releases of *P. persimilis* and *N. californicus* did suppress the spider mite population late in the season and the timing and number of predators released may determine their ecological impact, rather than the species or combination of species released (Ehler, 1992).

Steiner and Elliott (1983) recommended supplementing *P. persimilis* with *N. californicus* for the control of *T. urticae* on 'interior plantscapes' at higher temperatures. In orchards in Lerida, Spain, where an integrated mite management programme was put into place, the expense of using acaricides was cut by 71%; this was achieved with *N. californicus* and *A. andersoni* as the phytoseiid predators (Costa-Comelles *et al.*, 1990). When *T. urticae* was the only pest mite present on nursery citrus *N. californicus* reduced its population by >95% within 1-3 weeks of release (Grafton-Cardwell *et al.*, 1997) and it was found to give the most rapid control when compared with other predatory mite species.

5.2.2 *Panonychus* sp.

Neoseiulus californicus can consume all stages of *P. ulmi* (Bruhn & Beltrame, 1981). On apple trees in Chile, *N. californicus* consumed up to 18 mobile stages of *P. ulmi* every day for 3 days (Gonzalez, 1971). *Neoseiulus californicus* has a good searching capacity and has been found to eliminate *P. ulmi* from leaves with an average of 10 to 12 mobile stages on them. Gonzalez (1971) suggested that *N. californicus* is capable of keeping *P. ulmi* below an economic threshold of 6 spider mites per leaf, provided that the initial population of *P. ulmi* was not very high. *Neoseiulus californicus* has successfully colonised orchards in the south of France where its ability to control *P. ulmi* is particularly apparent in apple orchards prior to harvest. From 1989-1993, large numbers of the predator resulted in a reduction in the annual mean number of acaricide sprays from 2.5 to less than one (Fauvel *et al.*, 1993).

In Spain, *Panonychus citri* is an important pest of citrus trees. *Neoseiulus californicus* was present at high population levels, but only on trees where *P. citri* were abundant (Garcia-Mari *et al.*, 1983). However, a study by Garcia-Mari *et al.* (1991) on strawberry found the association between *P. citri* and *N. californicus* to be very low (18.7% compared to 45.6% with *T. urticae*). Steiner and Elliott (1983) found that for *P. citri*, *N. californicus* gave better control than *P. persimilis* for arboreal plant species. They suggested a rate of 20 predators/m² or two predators/plant (at a temperature of 21-27°C). On nursery citrus when the pest population consisted of only *P. citri*, *Euseius stipulatus* Congdon was found to be more effective in controlling the pest than *N. californicus*, and was able to maintain its density when the prey was absent (Grafton-Cardwell *et al.*, 1997).

5.2.3 *Oligonychus* sp.

Predation of *Oligonychus pratensis* (Banks) (Banks' grass mite) by *P. persimilis* and *N. californicus* under controlled laboratory conditions was examined by Pickett and Gilstrap (1986a). They found that *P. persimilis* reduced the grass mite population by 60%, whereas predation by *N. californicus* resulted in a reduction of 28%. The phytoseiids tended to feed on the instars that were the most abundant.

Oligonychus punicae (avocado brown mite) increases to moderate numbers on avocado trees in S. California (McMurtry *et al.*, 1984). Populations are usually adequately suppressed by

the coccinellid *Stethorus picipes* Casey, and the phytoseiid mite *Euseius hibisci* (Chant). However, occasionally populations reach >200 active stages per leaf and become a serious problem. A study was carried out in which different phytoseiid mites were used as potential predators to augment the natural predator population. Each of the potential phytoseiid predators was released on single-tree replicated plots, at a rate of 1200 mites/tree. *Neoseiulus californicus*, *Phytoseius macropilis* and *Metaseiulus occidentalis* (Nesbitt) were found to have the greatest numerical response to the prey population (McMurtry *et al.*, 1984).

Hoddle *et al.* (1999a) compared the efficacy of six phytoseiid species at controlling *Oligonychus perseae* Tuttle, Baker & Abbatiello, another tetranychid mite that is a pest of avocado. They found that *N. californicus* outperformed the other phytoseiids when *O. perseae* numbers were in a decline. They concluded that of the six species studied, *N. californicus* and *Galendromus helvoleus* (Chant) were the most promising for inoculative release. Both predators significantly reduced numbers of *O. perseae* when compared with oil-sprayed trees (standard control practice for *O. perseae*) and control (no treatment) trees (Kerguelen & Hoddle, 1999; Hoddle *et al.*, 1999b).

5.2.4 *Polyphagotarsonemus latus* (broad mite)

Under laboratory conditions *N. californicus* was shown to be a promising candidate for the biological control of *P. latus*. In comparison with data known for other phytoseiid mites feeding on this species, it has one of the shortest development times, low juvenile mortality and a favourable oviposition rate (Castagnoli & Falchini, 1993). The effect of predation of *N. californicus* and *Neoseiulus barkeri* Hughes on *Polyphagotarsonemus latus* infesting bean and lime (*Tilia* sp.) plants in the greenhouse and the field was examined by Peña and Osborne (1996). *Neoseiulus californicus* was capable of maintaining *P. latus* densities at low levels, whereas the effect of *N. barkeri* was inconsistent.

5.2.5 *Phytonemus pallidus* (Banks) (cyclamen mite)

N. californicus fed on *P. pallidus*, a pest on strawberry in North America, in the laboratory. However it was not as effective as *T. pyri* and *N. fallacis* which displayed greater levels of predation (Croft *et al.*, 1998b)

5.2.6 *Franklinella occidentalis* (western flower thrips)

A study carried out on predatory activity of some phytoseiid mites on *F. occidentalis* showed that the most effective species was *A. andersoni*, followed by *A. barkeri* and *N. californicus*. The fecundity of *N. californicus* feeding on eggs and first instar larvae of *F. occidentalis* was 1.0 and 0.52 eggs/day respectively, compared with 1.25 and 1.16 eggs per day for *A. andersoni* (Rodriguez-Reina *et al.*, 1992).

There has been little published on studies with the fruit tree red mite, *P. ulmi*, as the prey species. As *N. californicus* has been found on fruit trees, feeding studies were carried out to determine its potential as a biocontrol agent for *P. ulmi* and compare it with that for *T. urticae*. Thus, the aim of this chapter was to carry out a comparison of development rates and fecundity of *N. californicus* and *T. pyri* when feeding on either *T. urticae* or *P. ulmi*. *Typhlodromus pyri* were used as a means of comparison as they are the native predatory phytoseiid prevalent on apples in the UK. *Tetranychus urticae* were used as a prey comparison as both the predators will readily consume it.

5.3 Rearing methods

The rearing methods for *N. californicus*, *T. pyri*, *T. urticae* and *P. ulmi* are outlined below. These were used to produce large numbers of mites for all the experiments described in this thesis.

5.3.1 Culturing *N. californicus* and *T. pyri*

Culture plates were based on Overmeer's (1985a) rearing arena. A black plastic tile (11 x 22 x 2cm) was placed on a foam block (9 x 20 x 5cm). These were situated in a plastic container (16 x 28 x 9cm) which had a water reservoir in the bottom. Filter paper strips 1.5cm in width were placed around the edge of the black tile, and tissue was folded and placed on the strips such that it hung down into the water (Figure 5.1). Thus, the water reservoir kept the sponge and tissue wet, providing a water barrier around the arena. A sticky barrier (OecoTak A5, Oecos) was placed on the tissue to provide an extra boundary as many phytoseiids, including *N. californicus*, are very active and will run across the wet tissue. The OecoTak can be

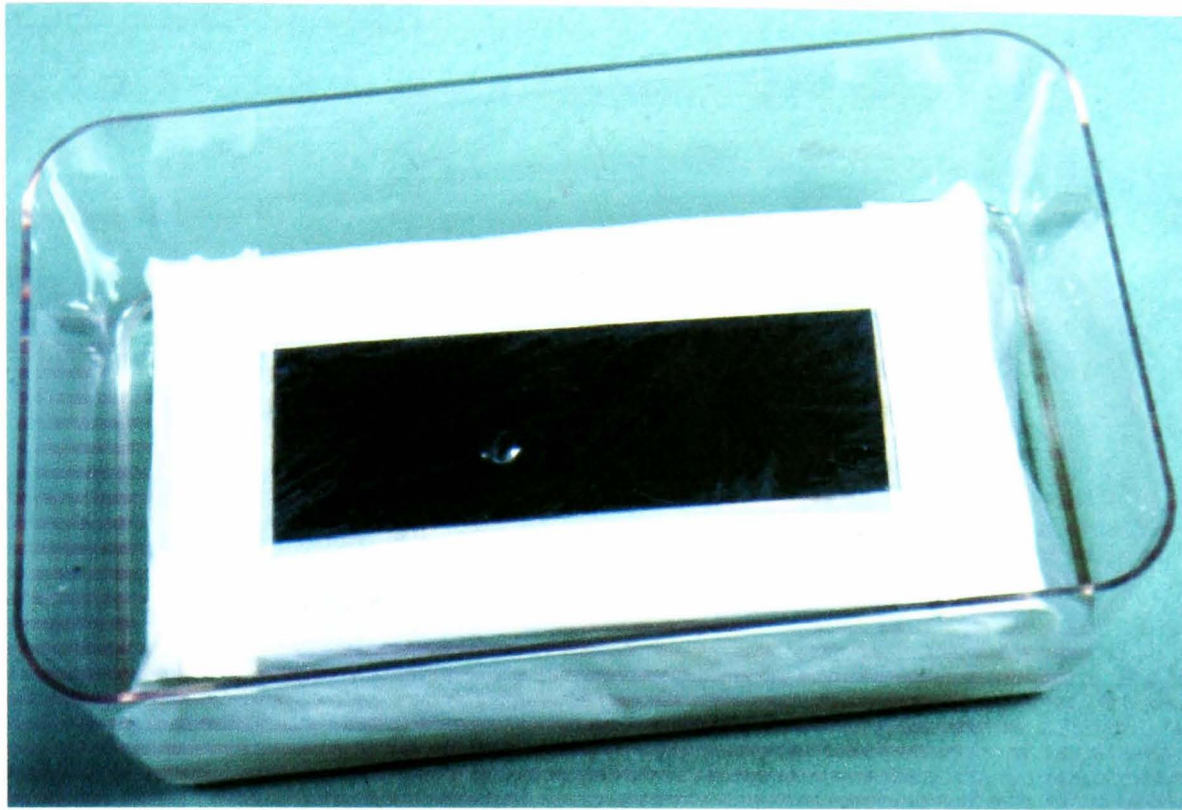


Figure 5.1 Culture plate for rearing predatory mites

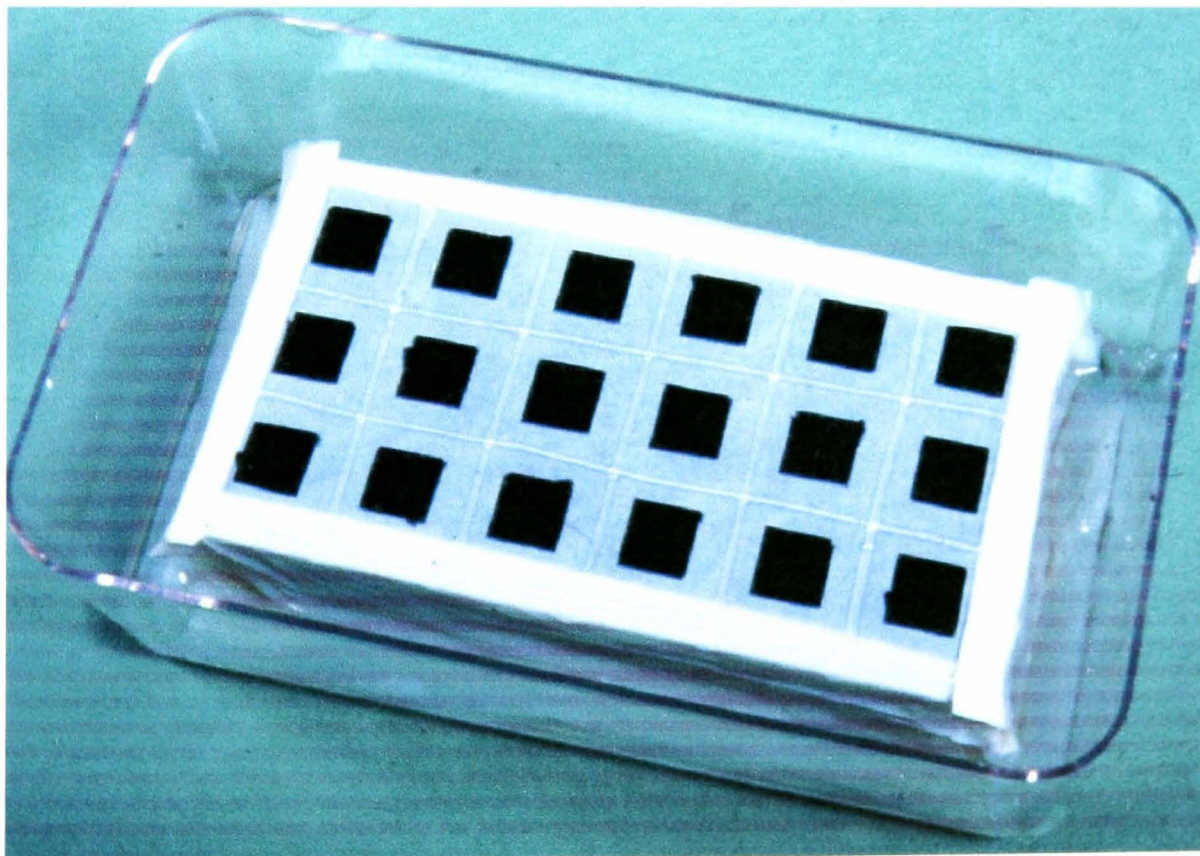


Figure 5.2 Culture plate for studying individual mites

applied with a large (10ml) syringe and this was aided by warming the full syringe in a beaker of hot water. A coverslip, supported by a few strands of cotton wool, provided shelter and an oviposition site for *T. pyri*. *Neoseiulus californicus* tended to oviposit around the edge of the culture plate alongside the filter paper, or inside small polyethylene capsules (BEEM capsules, size 3, Agar Scientific) that were placed on the plastic plate.

A culture was started by transferring mites with a fine sable artist's paintbrush (0000) onto the culture plate. The mites were fed broad bean (*Vicia faba*) pollen and *T. urticae* from a laboratory culture. The *T. urticae* were brushed off leaves with a large firm brush (38mm) onto a piece of paper, examined to check that only *T. urticae* were present, and then brushed onto the culture plates. A lid was placed on the *N. californicus* cultures as they fared better at higher humidity. However, a gap of a few centimetres was left otherwise the pollen and dead *T. urticae* quickly succumb to fungal growth. New culture plates were set up approximately every three months and the mites transferred.

5.3.2 Culture plate adapted for studying individual mites

The culture plate described above (5.3.1) was adapted into 18 individual arenas as follows: a metal template (11 x 22 x 2cm) was prepared in which 18 squares (1.9cm²) were cut out. This was used for cutting a large sheet of filter paper such that it was a rectangle of the appropriate size for the black tile and contained 18 individual arenas (Figure 5.2). Tissue was placed around the edges as before to form a water barrier and OecoTak was applied around all the squares so each arena was separated from the surrounding ones. A coverslip, supported by a few strands of cotton wool, was placed in each arena.

5.3.3 Culturing *T. urticae* and *P. ulmi*

Tetranychus urticae were cultured on dwarf French bean (*Phaseolus vulgaris*) plants. These were kept in a perspex cage with a gauze, removable front. The cage stood in a tray; sand surrounded the bottom of the cage to act as a barrier to mites walking off the plants. Pots containing French bean seeds were placed in the cage and germinated within. The culture was started by placing gravid female *T. urticae* onto the plants using a fine paintbrush.

Panonychus ulmi were cultured on apple trees and maintained in a glasshouse. These trees became contaminated with *T. urticae* and predatory mites. Therefore, when required for experiments the *P. ulmi* were moved individually using a fine paintbrush.

5.4 Consumption rates of *N. californicus* and *T. pyri*, feeding on *T. urticae* and *P. ulmi*

A preliminary experiment was carried out to determine consumption rates of *N. californicus* and *T. pyri* when fed on either *T. urticae* or *P. ulmi*. This was to ensure that the number of prey items made available during subsequent experiments would not fall short of the number that the predators would optimally consume.

5.4.1 Materials and methods

The *N. californicus* used were supplied by Koppert Ltd, Netherlands and had been originally collected in Spain. The *T. pyri* were from a culture that had been maintained in the laboratory for several years and were originally collected from an apple orchard at HRI, East Malling. Eggs were taken from these cultures and placed individually on leaf discs, 19mm diameter. The cultivar Greensleeves was used for the leaf discs as it has fewer leaf hairs and shed exuviae are, therefore, easier to find. The leaf discs were placed in small plastic containers (4x3cm pots, Kartell Plastics UK Ltd) onto capillary matting that covered the base (Figure 5.3). The capillary matting was kept wet, so that a water barrier surrounded the leaf disc. Ten prey items, either *T. urticae* or *P. ulmi* of immature stages (larvae, protonymph or deutonymph), were taken from laboratory cultures and placed onto the discs. Immature stages were used because *N. californicus* shows a preference for immature stages of *T. urticae* (Castagnoli & Amato, 1991; Mwambula, 1998). The plastic containers were placed within a larger plastic container (16 x 28 x 9cm) and a lid placed on top. The humidity within the box was approximately 95% and the temperature was $21 \pm 1^\circ\text{C}$; this was measured with a temperature logger (Tinytag Plus, Temperature range H (-30→+50°C) and relative humidity (0→100%RH)). The experiment was carried out at a photoperiod of 16L:8D. The leaf discs were examined twice a day, and the developmental stage of the predator and the number of prey individuals consumed was recorded. When a prey item was consumed a dried husk remained and could be counted, any missing prey were assumed to have walked off the leaf disc and into the water barrier. Any prey that had been eaten or had disappeared were replaced. The date and time at which each disc was examined was noted. Shed exuviae were

a confirmation that a mite had changed developmental stage. When these could not be found the stage was determined by an assessment of number of legs (larvae have only six), size and shape. Once mites had become adult they were mounted in PVA mountant on a microscope slide, cleared on a hotplate (49°C for 2h) and examined under a compound microscope. This was to check that they had indeed reached adulthood and that they were the species in question. The consumption rates were compared by two-way ANOVA.

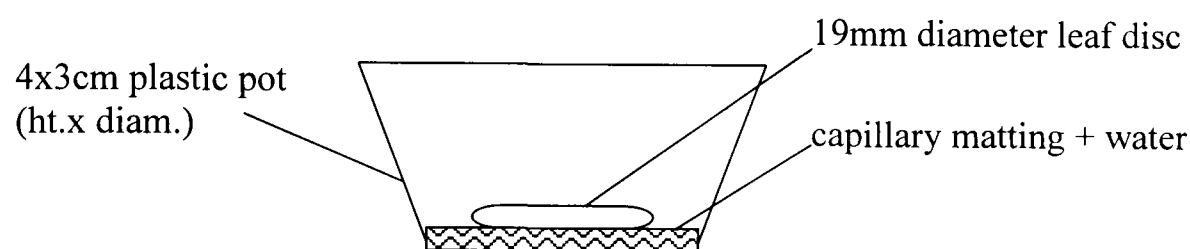


Figure 5.3 Diagram of experimental arena for studies involving predatory and phytophagous mites.

5.4.2 Results

The mean daily consumption rates of *N. californicus* and *T. pyri*, for each life stage feeding on either *T. urticae* or *P. ulmi* are shown in Table 5.1. In general, *N. californicus* consumed greater numbers of prey than *T. pyri*. The exception was adult *T. pyri*, which consumed greater numbers of *P. ulmi* immatures than *N. californicus* adults. The consumption rates of protonymphs were not significantly different, whereas those of deutonymphs and adults were significant ($p < 0.05$ and $p < 0.001$ respectively). The maximum number of prey items consumed per day by any stage of predator was 2.80.

Some *N. californicus* larvae were observed feeding and some had coloured guts. Phytoseiid mites have H-shaped guts which, when they feed on spider mite prey, are apparent as they

become coloured. For example, *N. californicus* that have fed on *P. ulmi* often have red guts and those that have fed on *T. urticae* often have green or brown coloured guts (personal observation). Although feeding by the larvae was observed, the number of prey that remained alive was the same. After moulting there appeared to be a period of time (approximately one day) during which the predators would be active but not feeding.

Table 5.1 Consumption rates (mean no. of individuals consumed per day) for each life stage of *N. californicus* and *T. pyri* when fed on either *T. urticae* or *P. ulmi*.

predator	prey	larva	mean no. of individuals consumed per day ± standard error				n (predators studied)
			protonymph	deutonymph	adult		
<i>N. californicus</i>	<i>P. ulmi</i>	0	1.58 ± 0.42	1.78 ± 0.22	1.17 ± 0.17		3
	<i>T. urticae</i>	0	1.95 ± 0.23	2.58 ± 0.39	2.42 ± 0.20		6
<i>T. pyri</i>	<i>P. ulmi</i>	0	1.25 ± 0.26	1.63 ± 0.18	2.80 ± 0.20		6
					(n = 5)		
	<i>T. urticae</i>	0	1.52 ± 0.29	1.40 ± 0.19	1.17 ± 0.17		5
					(n = 3)		

5.5 Development rates of *N. californicus* and *T. pyri*, feeding on *T. urticae* and *P. ulmi*

5.5.1 Materials and methods

The predatory mites used in this experiment were from the same cultures as before (section 5.4.1). Eggs were taken from these cultures and placed individually on leaf discs, 19mm diameter (cv. Greensleeves). The experimental details were as in 5.3.1. Ten prey items, either *T. urticae* or *P. ulmi* of immature life stages, were placed onto the discs. The leaf discs were examined twice a day, the developmental stage of the predator was recorded and any prey that had been eaten or had disappeared were replaced. Any data where readings were missed due to a weekend, or where the change in stage of a predator was in doubt, were

discounted. The results were inputted into Excel and a Visual Basic programme was used to read the data and determine the time spent at each developmental stage. The development times obtained were compared by multivariate ANOVA. The statistical analysis investigated the influence of predator type, predator sex and prey type on the development times of each immature stage.

5.5.2 Results

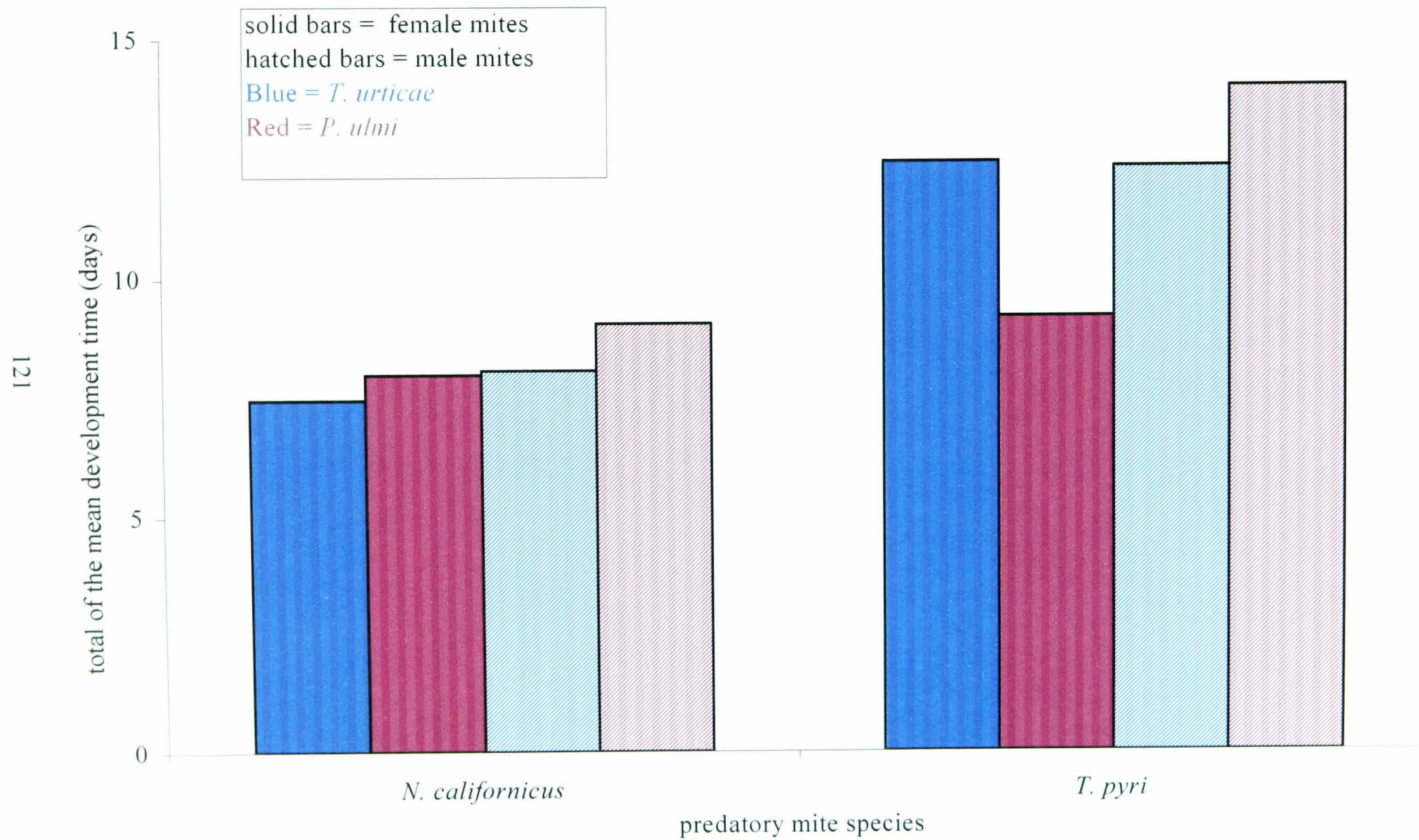
Neoseiulus californicus consumed both *P. ulmi* and *T. urticae* readily. Table 5.2 shows the mean development times for each stage of both male and female *N. californicus* and *T. pyri* when fed on the different prey types. Figure 5.4 is a bar chart of the total mean development times obtained.

There was a significant effect of sex on the development times of larvae ($p < 0.05$); in general female larvae developed quicker than male larvae. There were no other significant effects of prey type or predator type on larval development. For protonymphs and deutonymphs there was a significant effect of predator type on development times ($p < 0.001$), with *N. californicus* developing more quickly than *T. pyri*. There was also a significant effect of sex for protonymphs ($p < 0.05$) and an almost significant effect for deutonymphs ($p < 0.1$). In general, when comparing times for the same prey type, the development times for females was less than that for males. The exception was *T. pyri* feeding on *T. urticae* where the male development time was slightly faster for protonymphs and deutonymphs and about the same in total (from egg to adult). There was also an interaction between prey and sex, for both species of phytoseiid, ($p < 0.05$ for protonymphs and $p < 0.05$ for deutonymphs) and for deutonymphs there was an interaction between all three variables - predator, prey and sex ($p < 0.05$). Generally, *N. californicus* developed quicker than *T. pyri*; faster development times were obtained when *T. urticae* was the prey, and females developed faster than males.

Table 5.2 Development times for each stage of *N. californicus* and *T. pyri* when fed on either *T. urticae* or *P. ulmi* (16L:8D, 21±1°C, approx. 95%RH).

	sex	prey	mean time (days) ± standard error	mean development time (days) ± standard error (n)			total of the mean development times from egg to adult (days)
			egg	larva	protonymph	deutonymph	
<i>N. californicus</i>	female	<i>T. urticae</i>	2.42 ± 0.11	0.87 ± 0.08 (8)	1.89 ± 0.11 (9)	2.29 ± 0.25 (12)	7.47
		<i>P. ulmi</i>		1.17 ± 0.14 (7)	1.79 ± 0.44 (7)	2.62 ± 0.23 (10)	8.00
	male	<i>T. urticae</i>		0.90 ± 0.09 (11)	2.29 ± 0.16 (11)	2.47 ± 0.28 (14)	8.08
		<i>P. ulmi</i>		0.86 ± 0.13 (11)	2.82 ± 0.51 (12)	2.97 ± 0.20 (16)	9.07
<i>T. pyri</i>	female	<i>T. urticae</i>	2.86 ± 0.24	0.90 ± 0.06 (9)	3.87 ± 0.29 (9)	4.82 ± 0.70 (11)	12.45
		<i>P. ulmi</i>		0.87 ± 0.09 (4)	2.40 ± 0.09 (5)	3.08 ± 0.32 (6)	9.21
	male	<i>T. urticae</i>		1.23 ± 0.11 (10)	3.63 ± 0.24 (11)	4.62 ± 0.57 (11)	12.34
		<i>P. ulmi</i>		1.00 ± 0.10 (10)	4.14 ± 0.29 (10)	6.00 ± 0.72 (11)	14.00

Figure 5.4 Total of the mean development times from egg to adult for *N. californicus* and *T. pyri* fed on either *T. urticae* or *P. ulmi* (16L:8D, 21±1°C, approx. 95%RH).



5.6 Fecundity of *N. californicus* and *T. pyri*, feeding on *T. urticae* and *P. ulmi*

Unfortunately all the *P. ulmi* in culture died before this experiment could be completed and further supplies could not be obtained within the time remaining. In addition, the *T. pyri* that were used ran off the arenas into the sticky barrier and died. Results were obtained for fecundity of different strains of *N. californicus* but no direct comparison could be made between these and the fecundity of *T. pyri*. Therefore a brief comment is made here of the potential fecundity of *N. californicus* and it is dealt with in greater detail in Chapter 6.

5.6.1 Materials and methods

The *N. californicus* used were taken from cultures of different origins: Spain (Koppert Ltd. Netherlands), California (Nature's Control, USA), UK (East Malling, Kent collected from a strawberry plot). Eggs were taken from cultures and placed individually onto squares of a culture plate divided into 18 arenas separated by a sticky barrier (OecoTAK 05, Oecos) (Figure 5.2). The developing predators were fed *T. urticae* daily such that they were never short of food. The mites were examined once a day and once they had become adult the females were mated with males that had been fed the same prey. Once the female laid her first egg the male was removed from the arena, to avoid any potential effect of subsequent mating on fecundity. Although Castagnoli and Ligouri (1991) found that the number and size of endospermatophores had no correlation to either the time at which oviposition commenced or the total number of eggs produced, it was deemed best to be certain by removing the male. It was also important to ensure that mating was not disturbed as this would result in a reduction in the percentage of females that laid eggs, their fecundity and oviposition duration (Castagnoli & Ligouri, 1991). Females were examined once a day and the number of eggs that had been laid and the date and time were noted. The eggs were allowed to hatch, to ensure they were viable, and the larvae were removed from the arena. The females were observed until they died or, as in most cases, they ran off the arena and into the sticky barrier where they perished. The number of females for which fecundity data were successfully obtained was six Spanish, ten UK and 16 USA. Means were compared by single-factor ANOVA.

5.6.2 Results

The average fecundity was 0.80 eggs per day for the *N. californicus* from Spain, 0.82 eggs per day for those from the UK and 0.97 eggs per day for the USA strain. The difference in these means was not significant.

5.7 Discussion

Neoseiulus californicus readily consumed both *P. ulmi* and *T. urticae*, and in general consumed greater numbers of prey than *T. pyri*. No data, for the species used in this study, of prey consumption rates per day for individual mites have been published so a comparison cannot be made. Given that the maximum number of prey items consumed per day was 2.8, it would be optimal to ensure the availability of a greater number than three prey items at any time for feeding experiments. It should be noted that the numbers observed during the experiment were small, as it was designed as a preliminary study.

Neoseiulus californicus larvae were observed to feed on *T. urticae* prey and had coloured guts. However, zero consumption was recorded for larvae as no prey were killed. Therefore non-lethal feeding or feeding on alternative food sources, such as fungi present on the leaf discs, must have taken place. This supports the observation of Croft *et al.* (1999) that *N. californicus* have 'facultative feeding larvae'.

The apparent time lag, of approximately one day, between moulting and resuming feeding, during which the mite is active, is presumably because the sclerotised regions are hardening and during this period the mite is vulnerable and therefore needs to be mobile and does not risk feeding. Further studies in which mites are observed more frequently would establish with greater accuracy the period of time for which the mites do not feed. It would be interesting to also examine other species, as the author is unaware of any published observation of this sort.

Neoseiulus californicus developed quicker than *T. pyri* and this may give it an advantage over *T. pyri* as a biocontrol agent. Faster reproduction rates are characteristic of Type II predators (McMurtry & Croft, 1997) and in the field could be advantageous, allowing a quick response of predator numbers to an increase in prey population. In the present study the egg to adult

development times of *N. californicus* were 7.47 to 9.07 days (21±1°C, 95%RH, 16L:8D). Published data for development times of *N. californicus* on a range of prey are given in Table 5.4. Temperature and RH have a significant effect on development times and fecundity (Castagnoli & Simoni, 1991) and these should be taken into account when comparing published results, hence values for these are also shown in the table. It should be noted that, for most of these experiments, readings were taken every 24 hours so the mean egg to adult development times are likely to be approximate. The development times for the present study are comparable with those recorded by Castagnoli and Simoni (1991); their results at 21°C were 7.33 and 7.16 days. Their daylength was the same as in the present study and their humidity 20% lower. The results shown in Table 5.4 also show that there is, indeed, an effect of temperature and RH on development time and that prey type and possibly daylength should be taken into account also.

In the present study the egg to adult development times of *T. pyri* were 9.21 to 14.00 days (21±1°C, 95%RH, 16L:8D). Table 5.3 shows some published data for development times of this mite. The development times of the present study are comparable with those presented in the table. There is an effect of temperature on development time; Hayes and McArdle (1987) and Hardman and Rogers (1991) found the development time of *T. pyri* to be solely dependant on temperature.

Table 5.3 Published data for mean egg to adult development times for *T. pyri*. Daylength was 16L:8D in all cases.

temperature (°C)	relative humidity (%)	food source	egg to adult development time (days)		author
20	---	<i>Vicia faba</i> pollen	♀ 13.5-16.0	♂ 14.1-16.3	Fitzgerald, 1999
26-27	70-90	<i>P. ulmi</i>	6.61	6.41	Duso & Camporese, 1991
20	60	<i>P. ulmi</i>	14		Hayes & McArdle, 1987
18			14.77		
22	70	<i>P. ulmi</i>	11.80		Hardman & Rogers, 1991
26			8.96		
25	80	<i>T. urticae</i>	7 to 14 (depending on prey density)		Zhang & Croft, 1994

In the present study, female *N. californicus* developed quicker than males: this was also true for *T. pyri*. However, this was not the case for the results of Castagnoli and Simoni (1991) and Ma and Laing (1973) as can be seen from Table 5.4. The differences in development rates in these experiments may not reflect what happens in the field and may be an artefact of keeping the mites contained within experimental arenas.

Table 5.4 Published data for mean egg to adult development times for *N. californicus*. The values marked * are egg to egg times.

temperature (°C)	relative humidity (%)	daylength (L:D)	food source	egg to adult development time (days)		author
25	70	12:12	<i>T. urticae</i>	4.46		Mesa <i>et al.</i> , 1990
			<i>M. tanajoa</i>	4.71		
26	50	14:10	<i>T. urticae</i>	3.29		Friese & Gilstrap, 1985
			<i>T. cinnabarinus</i>	3.35		
			<i>T. urticae</i>	6.63		
25	90	16:8	<i>P. latus</i> (1 st generation)	6.08		Castagnoli & Falchini, 1993
			<i>P. latus</i> (2 nd generation)	7.32		
25	75	16:8	<i>T. urticae</i>	7*		Castagnoli <i>et al.</i> , 1999
25	75	16:8	<i>T. urticae</i> & <i>Quercus</i> sp. pollen	7.44*		Castagnoli <i>et al.</i> , 1995
				♀	♂	
13				22.28	21.83	
21				7.33	7.16	Castagnoli & Simoni, 1991
25	75	16:8	<i>T. urticae</i>	5.80	5.87	
33				3.80	3.78	
16.4	83-86			11.9	10.8	Ma & Laing, 1973
25	83-87	0:24	<i>T. urticae</i>	5.1	4.9	
32	86-92			3.9	4.0	

Neoseiulus californicus developed quicker on *T. urticae* than *P. ulmi*, therefore it is possibly better suited as a predator of *T. urticae*. They were fed on a mixture of *T. urticae* and *V. fabae*

pollen in culture and it is possible that this influenced the ability of the developing predators to assimilate *T. urticae*. However, it seems unlikely that maternal diet would affect the ability of hatched predatory mites to assimilate certain prey types. It is more likely that *T. urticae* are either easier to predate, possibly due to less defensive behaviour or being easier to locate, or that *T. urticae* provide a diet that encourages faster development.

Neoseiulus californicus laid between 0.8 and 0.97 eggs per day. This seems to be a low oviposition rate compared with published data. *Neoseiulus californicus* collected from citrus in the Mediterranean were found to have an ovipositional rate of 1.72 eggs per day when fed eggs and larvae of *Tetranychus pacificus* (McMurtry, 1977) (23.3°C, 16L:8D, RH not given). De Moraes and McMurtry (1985) carried out a study comparing *Tetranychus evansi* and *T. urticae* as prey for different phytoseiid mite species. *Neoseiulus californicus* displayed an oviposition rate of two eggs/female/day when feeding on *T. urticae* and 0.5 eggs/female/day when feeding on *T. evansi* (25°C, 40% RH, 12L:12D). Swirski *et al.* (1970) investigated the effect of prey-type on oviposition rate of *N. californicus*. The highest oviposition rates were achieved with *T. cinnabarinus* and *E. orientalis* as prey (2.37 eggs/day and 1.25 eggs/day respectively) (25-27°C, 60% RH, no daylength given). Swirski *et al.* (1970) also examined pollen as an alternative food source. The oviposition rate was highest for castor bean, and avocado (1.07-1.12 eggs/day), and moderate for maize and almond (0.88 and 0.81 eggs/day). It should be noted that the current study was carried out at a lower temperature than these experiments and that this may explain the lower oviposition rate, though the RH of the present study was higher and this has been shown to increase fecundity (Castagnoli & Simoni, 1991). Fitzgerald (1999) found the fecundity of different strains of *T. pyri* to vary from 0.58 to 0.73 eggs laid per day. This is slightly less than the fecundity for *N. californicus* in the present study.

In summary, the results obtained from the experiments described in this chapter indicate that *N. californicus* could be a suitable control agent of both *T. urticae* and *P. ulmi* as it consumes both readily and its development rates are faster than those of *T. pyri* (the native phytoseiid predaceous on these mites). However, further work would be needed to establish the full potential of this mite as a biocontrol agent for these pest species. Further studies could involve choice tests, olfactometer studies, examination of interactions and the effects of

competition between *N. californicus* and *T. pyri* and possibly electrophoretic or molecular testing of gut contents of field-caught predators.

CHAPTER 6: DIFFERENCES BETWEEN GEOGRAPHICALLY ISOLATED POPULATIONS OF *N. CALIFORNICUS*

6.1 Introduction

Early identifications of *Neoseiulus californicus* were made in California (McGregor, 1954; Schuster & Pritchard, 1963), southern France (Athias-Henriot, 1959; Schuster & Pritchard, 1963), Spain (McMurtry, 1977) and Chile (Dosse, 1958a; Gonzalez & Schuster, 1962). Thus, for at least 40 years, *N. californicus* has existed on different continents as geographically separate populations. A strain is a group of organisms within a species that is distinguished by one or more minor characteristics (Hale & Margham, 1988). *Neoseiulus californicus* were obtained from Spain (Koppert Ltd, Netherlands), California (Nature's Control, USA) and the UK (collected from a strawberry plot at HRI, East Malling, Kent). The aim of the work described in this chapter was to investigate morphological, biological and biochemical characteristics of these three groups of mites in an attempt to detect any characteristics that would indicate distinct strain differences. If different strains were present an answer might also be obtained as to the question of the origin of the UK mites.

6.2 Cross-mating experiment

6.2.1 Materials and methods

Eggs were taken from culture plates of *N. californicus* from the UK, Spain and the USA. The origin of the mites was the same as described in the introduction above (6.1). Each individual egg was placed on a square of a culture plate divided into 18. The egg was allowed to develop to adult, and females were mated with males from different origins. All the mites were fed a mixture of *Tetranychus urticae* and *Vicia faba* pollen. Between two and seven pairings were carried out for each combination of cross (see Table 6.1). Female mites were examined once a day and mating, gravid state and egg laying were noted. Once the first egg was laid the males were removed from the square. Values for fecundity were obtained by monitoring the number of eggs laid over a period of approximately ten days. The eggs were left to hatch to check viability. Many females ran out of the arena and into the sticky barrier and perished, and for these data could not be obtained.

6.2.2 Results

All the pairings were successful, resulting in gravid females which laid viable eggs; the results are shown in Table 6.1. The self-crosses (i.e. mites of the same origin) resulted in fecundities of 1.40, 1.34 and 1.32 eggs per day for USA, Spain and UK respectively. These were greater than the fecundities of the other crosses. The remaining crosses resulted in fecundities ranging from 1.14 to 0.61 eggs per day. The amount of data obtained was not sufficient to apply a statistical analysis, as some of the crosses had only two or three pairings. Few females survived for 10 days in all the pairings, resulting in insufficient egg-laying data for fecundity analysis. The fecundities recorded in this experiment were greater than those in chapter 5, section 5.5.

Table 6.1 Cross mating of *N. californicus* from different countries. The numbers of successful matings and the resulting fecundity are given.

♀ origin	♂ origin	no of pairs examined	no. successfully mated (resulting in gravid females)	mean no of eggs laid per day
USA	USA	2	2	1.40
USA	Spain	5	5	1.14
USA	UK	6	6	0.84
Spain	Spain	3	3	1.34
Spain	USA	4	4	0.61
Spain	UK	5	5	0.67
UK	UK	5	5	1.32
UK	Spain	7	7	0.93
UK	USA	4	4	1.08

6.3 Measurements of morphological characteristics

6.3.1 Materials and methods

Twenty female mites were taken from each culture (Spain, USA and UK). The cultures had been maintained in the same conditions (21°C, 16L:8D) (see chapter 4, section 4.1) and for a similar period of time (between one to two years). They were mounted on microscope slides in polyvinyl alcohol (PVA) mountant and cleared on a hotplate (49°C for 2h). The mites were examined with a compound microscope and measurements were taken using an eyepiece graticule. The eyepiece graticule measurements were calibrated with a stage graticule. The morphological features measured were: length and width of dorsal and ventrianal shields, length of the macroseta on leg IV and length of setae J2 and Z5. The shields and macroseta were selected as these are the structures often measured for taxonomic purposes. The setae were selected for the reasons stated previously (Chapter 2, section 2.5); J2 is a mid-dorsal seta and Z5 a margino-dorsal seta (Sabelis & Bakker, 1992).

The results were analysed by ANOVA, correlation matrices and canonical variate analysis (CVA). ANOVA ignores correlation between variates (each morphological characteristic measured) and just examines the differences between the 'strains' (the mites from the three different origins). Thus, it was important to also look at the levels of correlation between the morphological characteristics. For example, is dorsal shield length correlated with dorsal shield width? A correlation matrix looks at the correlation between pairwise variates. To combine ANOVA and correlation effects a multivariate analysis was required. CVA maximises the ratio of between group variation to within group variation to find measures that discriminate between pre-determined groups of data.

6.3.2 Results

The mean measurements of a selection of morphological characteristics for the three possible strains of *N. californicus* are represented in Table 6.2. There appears to be a general trend of size increase with mites from the UK being the smallest and those from the USA the largest.

The ANOVA showed significant differences between 'strains' for all morphological characteristics measured ($p < 0.01$) for all except ventrianal shield width ($p = 0.005$) and seta J2 ($p = 0.05$). To discriminate between each 'strain', an approximate t-test (difference between

means of more than twice the s.e.d. implies significance at $p=0.05$) showed that all the 'strains' had significantly different means for the variates dorsal shield width and seta Z5. The UK 'strain' had significantly different means from the USA and Spanish 'strains' (which were not significantly different from each other) for dorsal shield length, ventrianal shield length, ventrianal shield width and macroseta on leg IV. The Spanish 'strain' had significantly different means from the UK and USA 'strains' for seta J2.

Table 6.2 Mean lengths of selected morphological characteristics for different 'strains' of *N. californicus*. Standard error and standard error of difference of means (s.e.d.) are given.

	mean length (μm) \pm standard error of <i>N. californicus</i> 'strain' from:			s.e.d.
	HRI-EM, UK	Valencia, Spain	California, USA	
dorsal shield length	345.09 \pm 1.72	330.67 \pm 1.93	328.55 \pm 2.37	2.89
dorsal shield width	180.83 \pm 1.55	176.39 \pm 1.44	167.90 \pm 1.79	2.27
ventrianal shield length	116.96 \pm 1.03	111.46 \pm 0.94	110.75 \pm 1.34	1.59
ventrianal shield width	102.80 \pm 0.99	99.25 \pm 0.98	97.31 \pm 1.42	1.64
leg IV macroseta	46.56 \pm 0.91	42.09 \pm 0.66	43.88 \pm 0.60	1.03
seta J2	25.75 \pm 0.56	24.24 \pm 0.46	26.65 \pm 0.35	0.65
seta Z5	64.62 \pm 0.93	59.80 \pm 0.57	62.28 \pm 0.74	1.07
n	19 (except seta J ₂ n=18)	20 (except leg IV macroseta n=19)	20	

The correlation matrix and matrix scatterplot for all the samples together are shown in Figure 6.1. For all the mites there was no correlation between seta J₂ and any of the other variates, and there was little correlation between Z₅ and the other variates (where any correlation occurred it was with one of two of the dorsal or ventrianal shield characteristics). All the rest of the variates correlated significantly at the $p<0.001$ level, except for dorsal shield width + Z₅ and ventrianal shield width + Z₅ which was significant at the $p<0.01$ level. The correlation

(a)

*** Degrees of freedom ***

Correlations: 55 (all samples)

*** Correlation matrix ***

dsl	1	1.000							
dsw	2	0.772	1.000						
vsl	3	0.810	0.739	1.000					
vsw	4	0.668	0.590	0.622	1.000				
leg4	5	0.538	0.395	0.523	0.436	1.000			
J2	6	0.028	-0.186	-0.080	-0.082	0.157	1.000		
Z5	7	0.475	0.334	0.458	0.365	0.454	0.060	1.000	
		1	2	3	4	5	6	7	

(b)

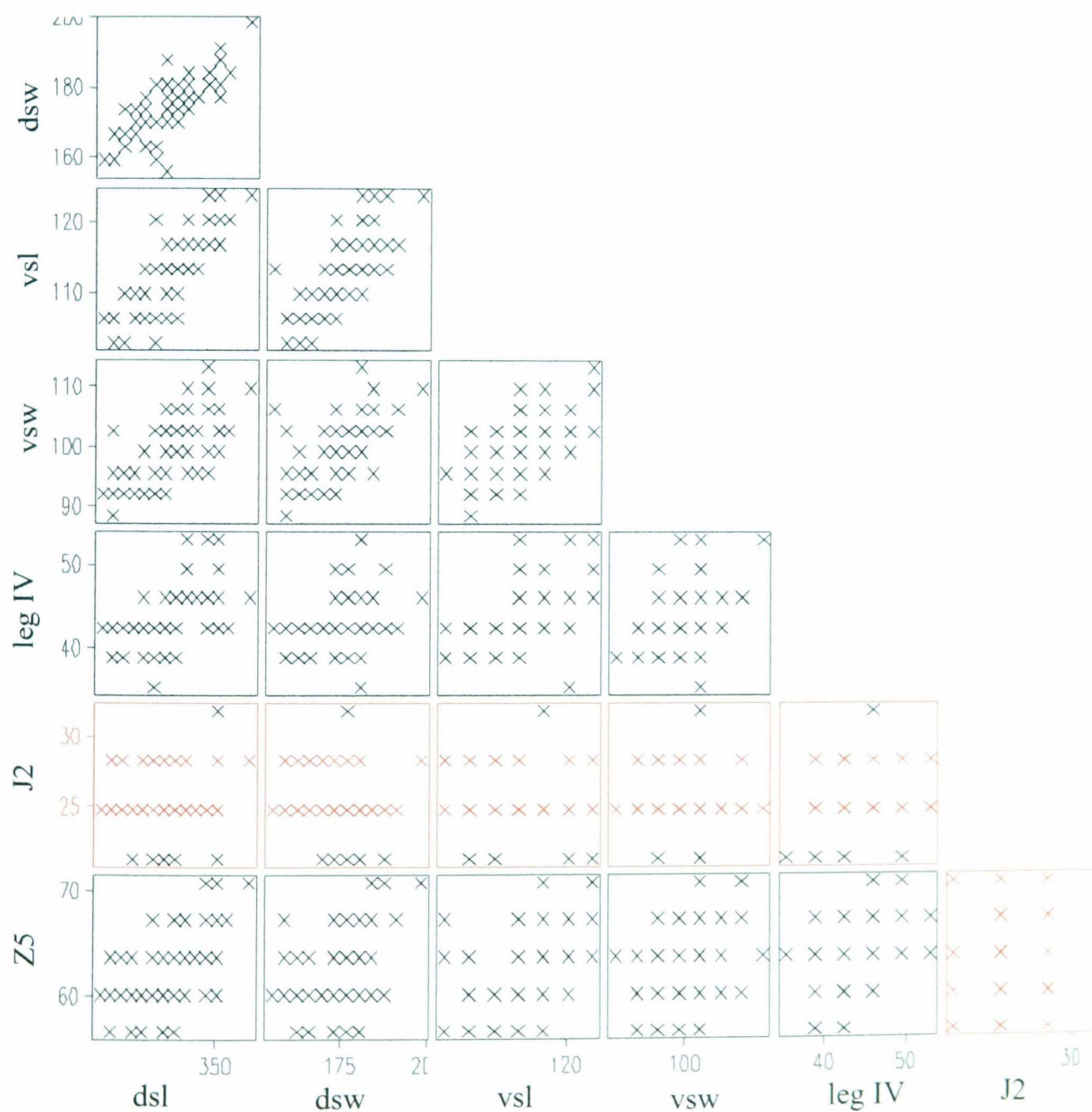


Figure 6.1 Correlation matrix (a) and matrix scatterplot (b) for all the samples (mites from the three different origins) together. Pairs of variates with no correlation are highlighted in red

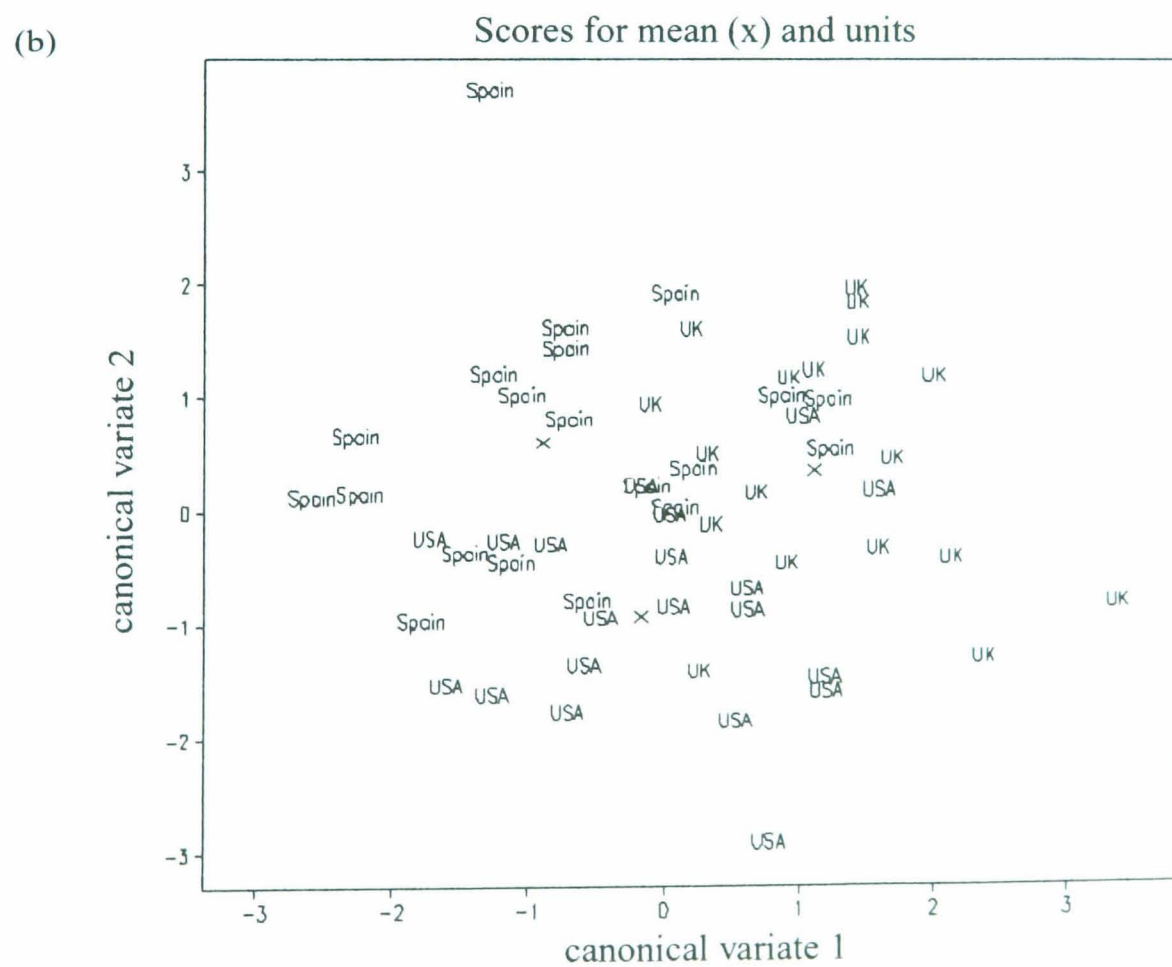
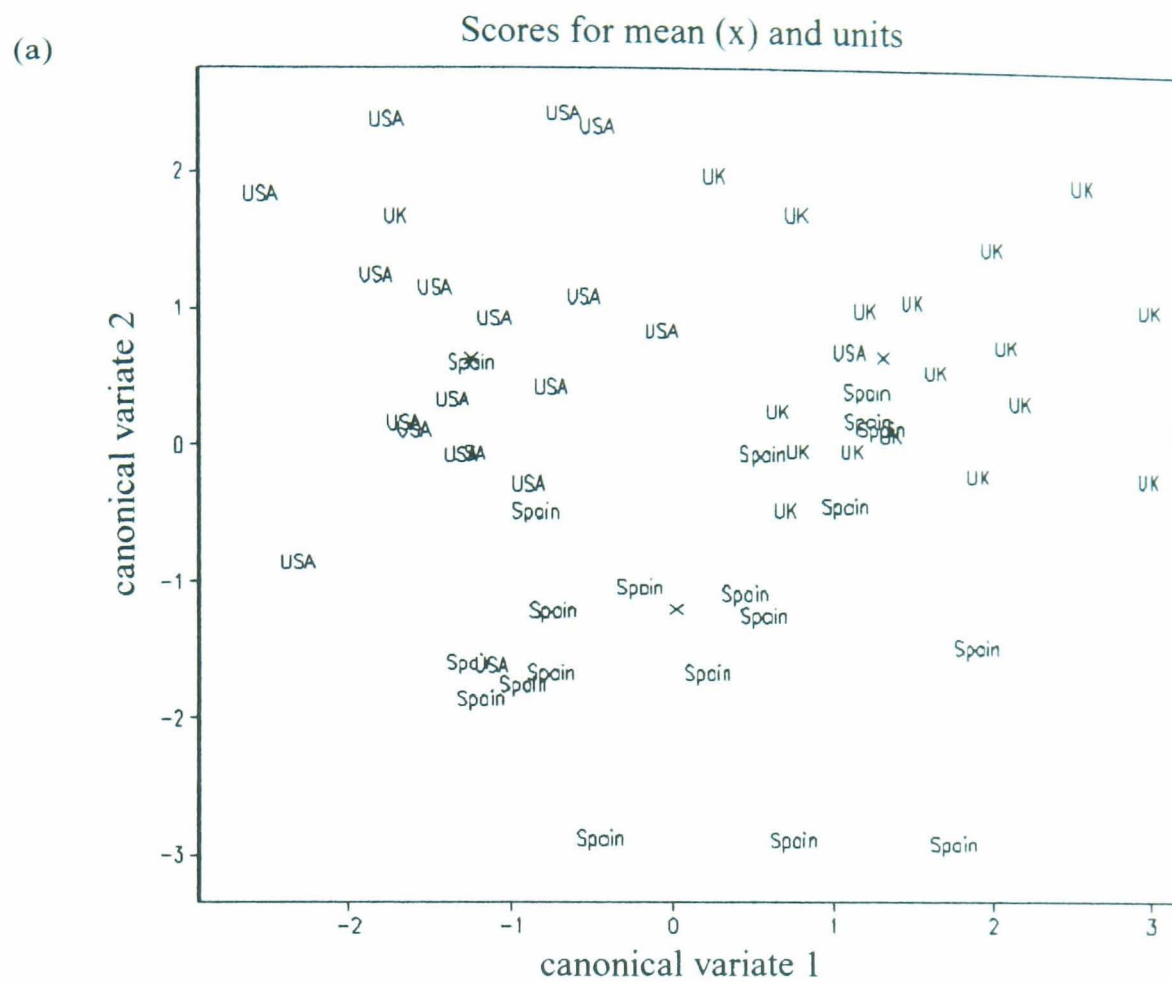


Figure 6.2 CVA Scatterplots for a combination of all seven variates (a) and a combination of shield measurements only (b)

matrices for the individual ‘strains’ were all similar to that of all the samples together, except that for the USA and UK mites the leg IV macroseta did not correlate with any of the other variates, but for the Spanish samples it did ($p=0.05$).

The CVA showed that a combination of all seven variates gave better discrimination than a combination of just the dorsal and ventrianal shield measurements. Figure 6.2 shows scatterplots of the CVA for these two possible combinations. It can be seen that the scatterplot for a combination of all seven variates distributes the ‘strains’ into more distinctly separate groups than that for the shield measurements. There is, however, some overlap and completely separate categories cannot be discerned.

6.4 Development rates

6.4.1 Materials and methods

The *N. californicus* used were taken from the cultures of mites from the different origins (Spain, USA and UK). Eggs were taken from these cultures and placed individually on leaf discs, 19mm in diameter. The cultivar Greensleeves was used for the leaf discs as it has fewer leaf hairs and shed exuviae are therefore easier to find. The leaf discs were placed in small plastic containers (4x3cm pots, Kartell Plastics UK Ltd) onto capillary matting which covered the base (see Chapter 5, Figure 5.3). The capillary matting was kept wet, so that a water barrier surrounded the leaf disc. Ten prey items, either *T. urticae* or *Panonychus ulmi* of immature life stages, were placed onto the discs. The leaf discs were examined twice a day, the developmental stage of the predator was recorded and any prey that had been eaten or had disappeared (occasionally mites walked off the disc and into the water barrier) were replaced. The date and time at which each disc was examined was noted. The presence of shed exuviae were a confirmation that a mite had changed developmental stage. When these could not be found, stage was determined by an assessment of number of legs (larvae have only six), size and shape. Once mites had become adult they were mounted in PVA mountant on a microscope slide, cleared on a hotplate (49°C for 2h) and examined under a compound microscope. This was to check that they had indeed reached adulthood, their sex and that they were the species in question. The development times were compared by single-factor ANOVA.

6.4.2 Results

The results were entered into Excel and a Visual Basic programme was used to assess the data and determine the time spent at each developmental stage. The mean development times for each immature stage and the total egg hatch to adult times are presented in Table 6.3. American *N. californicus* showed the fastest total development times and UK *N. californicus* the slowest. *Neoseiulus californicus* males from the USA had the quickest total (egg hatch to adult) development time of 6.08 days. UK females were slowest, with a development time of 8.04 days. In all 'strains' males developed faster than females. There were no significant differences between the development times of larvae or deutonymphs, but there were significant differences between the development times of the protonymphs from different origins ($p < 0.001$). There was also a significant difference in the total development times ($p < 0.01$).

Table 6.3 Mean development times for *N. californicus* from different origins.

Origin of <i>N.</i> <i>californicus</i>	sex	mean development time (days) \pm standard error (n)			
		larva	protonymph	deutonymph	total (egg hatch to adult)
UK	f	1.40 \pm 0.09 (11)	3.34 \pm 0.20 (13)	3.24 \pm 0.31 (13)	8.04 \pm 0.58 (11)
	m	1.34 \pm 0.10 (6)	2.72 \pm 0.23 (8)	2.31 \pm 0.17 (8)	6.43 \pm 0.26 (6)
Spain	f	1.50 \pm 0.22 (4)	3.48 \pm 0.40 (6)	2.65 \pm 0.27 (6)	6.93 \pm 0.60 (4)
	m	1.23 \pm 0.11 (5)	2.30 \pm 0.12 (5)	2.62 \pm 0.32 (5)	6.15 \pm 0.35 (5)
USA	f	1.40 \pm 0.09 (15)	2.72 \pm 0.16 (17)	2.62 \pm 0.17 (17)	6.53 \pm 0.23 (15)
	m	1.26 \pm 0.07 (13)	2.24 \pm 0.15 (14)	2.73 \pm 0.14 (14)	6.08 \pm 0.20 (13)

6.5 Fecundity

6.5.1 Materials and methods

The *N. californicus* used were taken from the three cultures of different origins as mentioned previously in this chapter (section 6.2). Eggs were taken from these cultures and placed individually onto squares of a culture plate divided into 18 arenas separated by a sticky barrier (OecoTAK 05, Oecos). The developing predators were fed *T. urticae* and *V. faba* pollen daily, such that they were never short of food. The mites were examined twice a day and once they had become adult the females were mated with males that had been fed the same prey. Once the female laid her first egg the male was removed from the arena, to avoid any potential effect of subsequent matings on fecundity. Although Castagnoli and Ligouri (1991) found that the number and size of endospermatophores showed no correlation to either the time at which oviposition commenced or the total number of eggs produced, it was considered prudent to be certain by removing the male. It was also important to ensure that mating was not disturbed as this would result in a reduction in the percentage of females that laid eggs, fecundity and oviposition duration (Castagnoli & Ligouri, 1991). Females were examined twice a day and the number of eggs that had been laid and the date and time were noted. The eggs were allowed to hatch, to ensure they were viable, and the larvae were removed from the arena. Any prey eaten were replaced. The females were observed until they died or, as in most cases, they ran off the arena and into the sticky barrier where they perished. The data for fecundity were analysed by single-factor ANOVA.

6.5.2 Results

The fecundity of *N. californicus* ranged from 0.80 eggs per day to 0.97 eggs per day. The mean fecundity for each of the different 'strains' of *N. californicus* is shown in Table 6.4. The Spanish mites exhibited the lowest fecundity and the American ones the greatest, but the differences were not statistically significant.

Table 6.4 The fecundity of different 'strains' of *N. californicus*.

	origin of <i>N. californicus</i> 'strain'		
	UK	Spain	USA
mean no. of eggs laid/day	0.82	0.80	0.97
standard error	0.13	0.13	0.11
maximum	1.69	1.24	1.77
minimum	0.30	0.42	0.38
n	10	6	16

6.6 Electrophoretic technique for distinguishing strain differences

6.6.1 Materials and methods

The cultures of *N. californicus* were fed a mixture of *T. urticae* and *Vicia faba* pollen. The pollen does not produce any bands on these gels (personal observation). Thrips (*Thrips* sp.) occasionally infested the plants that the *T. urticae* were cultured on and were sometimes found on the phytoseiid culture plates also. Thus, thrips and *T. urticae* were also prepared for electrophoresis to compare with the bands produced from non-starved predatory mites.

Mites were taken from cultures and squashed in micro-maceration tubes (Murray & Solomon, 1985) in 10µl sample buffer (see Appendix VII). Samples were frozen (-20°C) immediately, and maintained in the freezer until they were used for electrophoresis. Before applying samples to wells in the gel, a drop of 0.05% bromophenol blue was added to each as an indicator. Samples were applied and the gel was run at 120V for 30 minutes or until the samples had reached the end of the stacking gel. This could be seen by the position of a blue band (from the bromophenol blue) on the gel. The voltage was then increased to 140V and the gel run until the front reached a few millimetres from the bottom. The gel was removed and rinsed in distilled water and stained in the dark to reveal bands with esterase activity. Esterase staining was chosen because this is capable of higher sensitivity than many other stains (Murray *et al.*, 1989). The gel stain recipe is given in Appendix VII.

Electrophoresis was carried out on vertical polyacrylamide native gels, 1mm thick, which were run in a Mini Protean II dual slab cell tank (Bio-Rad). The gels were discontinuous with a 7.5% separating gel (0.375M Tris, pH8.8) and a 4% stacking gel (0.125M Tris, pH6.8). The gel and buffer recipes are given in Appendix VI. The wells were produced with a 15-well comb, 0.75mm thick. The wells were 3mm across and had the capacity to hold 16µl of liquid.

6.6.2 Results

Examples of three electrophoresis gels are shown in Figure 6.3. *Neoseiulus californicus*, *T. pyri*, *T. urticae* and thrips all gave distinctive band patterns on the gels. *Neoseiulus californicus* produced three major bands, with a distinctive dark band around the middle of the gel and two others in the bottom half of the gel. This can be easily seen in gel 1, well numbers 5 to 10. Thrips produced a pattern of three bands close together around the middle of the gel, and then a fourth further down. This can be seen in gel 1, well 2. *Typhlodromus pyri* produced a very faint pattern of bands, just discernible in gel 3, wells 1 and 2. *Tetranychus urticae* produced a pattern of bands that look like a streak down the gel. *Tetranychus urticae* were in well 15 of gel 1, well 13 of gel 2 and well 11 of gel 3.

Gel 1 shows band patterns for *N. californicus* from the three different origins. The USA 'strain' were in wells 3 to 6, the Spanish 'strain' in wells 7 to 10 and the UK ones in wells 11 to 14. The samples increased in concentration from left to right, with samples of 1, 2, 5 and 10 mites respectively. The intensity of the bands increased with sample concentration.

The band pattern of mites from USA and Spain appear similar, though the lower bands appear in different places on the gel. Those from the UK appear to have fewer bands in the bottom half of the gel and the large band in the central area seems to be made up of at least two separate bands. To investigate this further, gels 2 and 3 were run with dilutions of predatory mite material to attempt to clarify the number of bands in this middle region.

Gels 2 and 3 show the same pattern of bands for *N. californicus* as gel 1. For each group of mites dilutions from left to right were: 5, 2, 1, 0.2, 0.1 mite. The USA 'strain' were in wells 1 to 5 and the Spanish 'strain' in wells 7 to 11. These show the same pattern of bands as before

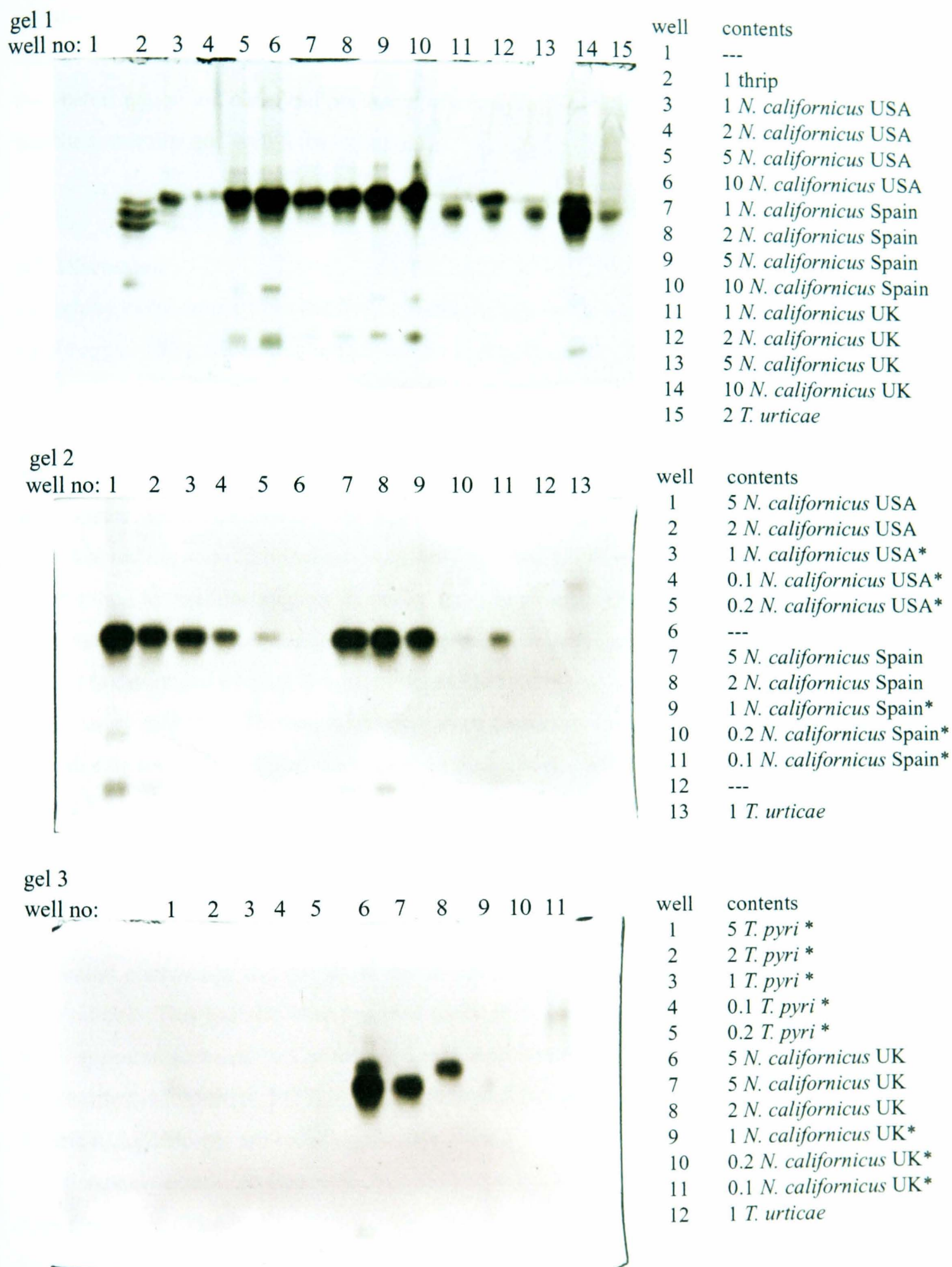


Figure 6.3 Examples of three electrophoresis gels stained for esterase activity. Well numbers are shown above the gels and a table of the corresponding samples displayed next to each. Those marked with a * were starved for 24h before maceration, and were serial dilutions.

and the 0.2 and 0.1 dilutions still gave only one band in the central region of the gel. The UK 'strain' of *N. californicus*, in gel 3, were in wells 6 to 10. There appears to be two bands in the central region but these did not occur in every sample. Well 6 had both, whereas well 7 had the lower one and well 8 the upper one.

6.7 Discussion

Neoseiulus californicus from the three different origins were successfully crossed, producing viable eggs. There has been one previous cross-breeding study, which involved crossing *N. californicus* from California with those from Chile and Peru (McMurtry and Badii, 1989). This resulted in male and female progeny and they suggested that the absence of reproductive barriers between separated populations of *N. californicus* showed either a recent movement to geographical areas or a relative stability of morphological and/or physical characteristics related to mating and reproduction (McMurtry and Badii, 1989). They noted that this is not true for the Phytoseiidae in general; studies on *Euseius* sp. have indicated the existence of numerous strains with varying degrees of reproductive incompatibility. Further work is needed to determine whether the differences in fecundity that resulted (Table 6.6) are significantly different. This would involve more crosses (ten of each would have provided enough data for statistical analysis), and then back-crosses with the progeny. It would have also been necessary to rear the resultant offspring of both the F₁ and F₂ generations through to adult to check that they were fertile also.

Examination of *N. californicus* from the three different geographical origins under a compound microscope did not show any morphologically distinguishable features (personal observation). This has also been reported previously; *N. californicus* collected from citrus in Spain appeared identical to Californian specimens which had been collected from citrus and strawberries (McMurtry, 1977). *Neoseiulus californicus* specimens from southern Europe, Algeria and California are indistinguishable from *N. chilensis* from Chile (McMurtry, 1977 - by personal communication with Athias-Henriot). Specimens from Guatemala, Peru and Argentina were examined and no 'consistent differences' between them and specimens from Spain or California were observed (McMurtry, 1977). Thus, the current study concentrated

on determining whether there were significant differences in the size of certain morphological characteristics.

Previous studies have been unable to show consistent differences between morphological measurements from mites collected from different countries or plants. Examination of specimens of Californian *Neoseiulus californicus* and populations of *Neoseiulus chilensis* from either Chile or Peru showed no consistent differences in dorsal shield setal measurements, spermatheca shape, ventrianal shield characteristics and leg macroseta length (McMurtry and Badii, 1989). Female *A. californicus* collected in Peru showed slight differences in the length of some dorsal setae (the particular setae were not noted). However, there were no significant morphological differences in any other characters measured (El-Banhawy, 1979).

The present study found significant differences between 'strains' for all variates measured, except seta J₂, and this characteristic did not correlate with any of the other variates. It is possible that this seta is sufficiently small that not enough variation in measurements was gained with the graticule used. The statistical analysis showed that there are significant differences between the measurements of the mites from the three different geographical origins. However, no single measurement can be used to distinguish the 'strains' and for all the variables there was overlap between the groups of mites (as demonstrated by the CVA scatterplots). The significant differences in size of the morphological characteristics obtained may be an indication that different strains of *N. californicus* exist. However, as a means of identifying strains this method is not recommended as individual field-captured mites could not be assigned to a distinctive size category.

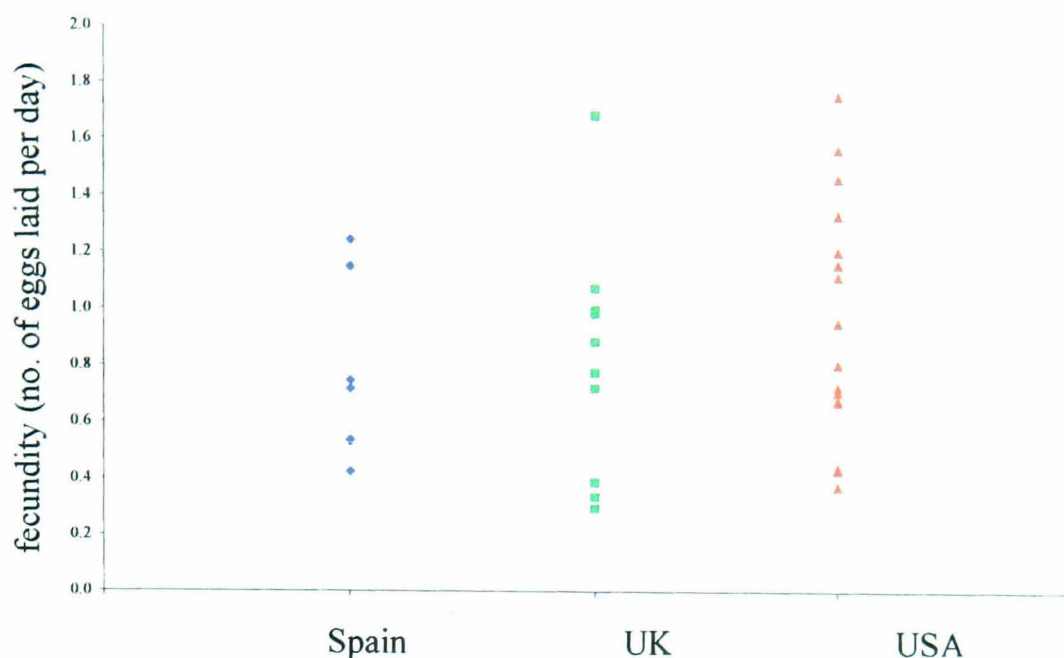
Unlike the previous study on the development of *N. californicus* when fed either *T. urticae* or *P. ulmi* as prey (see chapter 5, section 5.4), male *N. californicus* developed faster than females *N. californicus*. This was also true for the results of Castagnoli and Simoni (1991) and Ma and Laing (1973) in their development studies of *N. californicus* feeding on *T. urticae* (see chapter 5, Table 5.3). Females might have a longer development time as they have to develop the reproductive structures necessary for egg production. There were significant differences in the total development times, with the American *N. californicus* developing quickest and the

UK ones the slowest. This may be an indicator of their relative potential 'fitness' and hence biocontrol potential. Alternatively an advantage of slower development times might be that increased feeding times at each developmental stage would result in stronger and fitter individuals and that, as a non-native species, the *N. californicus* in the UK have fewer predators than in their native countries and therefore there is no evolutionary pressure for fast development times.

The difference between the maximum and minimum values for fecundity was large and it is useful to see whether these are outliers or whether the data are ranged between these values. Figure 6.4 is a scatterplot showing the values for fecundity obtained for each mite studied. It appears that the values are spread between the maximum and minimum points. The possible exception is the maximum value for the UK; however, the number of results obtained in total is rather small and further experiments may show that this is not an outlier. In general, it seems that females exhibit a range of fecundities and this would explain why no statistically significant differences were found. It should be noted that the numbers of individuals for which data were obtained was low and that ideally this experiment should be repeated to gather further data.

The intensity of bands on the electrophoresis gels corresponded to the amount of mite material in the original sample. With the exception of the dark bands in the middle of the gel, it is necessary to have a sample size of approximately 5 mites to be able to discern all the bands present. For *T. pyri* it is necessary to use at least this number as the bands produced are very faint. This was a disadvantage of this type of electrophoresis as individual mites did not show up enough bands to determine a variation in pattern. This is not true for electrophoresis in general; Fitzgerald (1999) obtained easily discernible bands for *T. pyri* with gradient gels.

Figure 6.4 Scatterplot showing the spread of results obtained for fecundity of *N. californicus* of different origins



The *T. urticae* and thrips produced very different band patterns to those of the predatory mites. If the predators had fed upon these prey items it did not have much impact on the pattern of bands produced. The lowest thrips band is in a similar position on gel 1 to a USA *N. californicus* band. However, they are not at exactly the same position and it can be concluded that they represent different esterases. Repetition of this experiment with large numbers of starved predators would confirm that the USA band belongs to the predatory mite and was not of prey origin.

All three groups of mites show different band patterns. The USA and Spanish mites appear to give similar band patterns, although the lower bands appear in slightly different places on the gel. The UK 'strain' has similar lower bands, with the upper of the two corresponding to the Spanish mites and the lower corresponding to the USA ones. The main difference between the UK mites and the others is that the large dark band in the middle of the gel appears to consist of two bands, both of which are not always present. There is potential for different strains to be distinguished by electrophoresis as the different groups of mites show different patterns. However, the different position of the lower bands only becomes apparent when the

samples are run on the same gel. If a mite was taken from the field and run out on a gel it would be difficult to determine which group it matched.

The difference in band patterns could be caused by previous pesticide exposure. Esterases can be involved in pesticide resistance. For example, organophosphorus compounds are potent inhibitors of the active site of acetylcholinesterase. They stop the acetylcholinesterase hydrolysing acetylcholine which 'resets' the nerve synapse after a nerve impulse has passed across, and thus cause paralysis. The UK mites were collected from a plot that had been treated, a week previously, with the pyrethroid bifenthrin (Talstar). Esterases play a part in pyrethroid resistance also; this may be direct ester cleavage or sequestering of the pyrethroid (Cahill *et al.*, 1995). Thus, staining for esterases may show up differences caused by pesticide exposure as susceptible mites will have less or an absence of certain esterases and resistant ones will have greater quantities, thus giving different band patterns.

The findings of the present study do not point unequivocally to the UK strain having originated from either Spain or the USA. The USA strain were represented by the smallest mites, but had the fastest development rates and (although not statistically significant) the greatest fecundity. The Spanish strain showed a lower fecundity and development rate and were larger than the USA mites but smaller than the UK ones. The UK strain displayed the slowest development rates and similar fecundity to the Spanish mites. Thus, the response of the UK strain to the morphological and biological experiments indicated its similarity to the Spanish strain. However, in the diapause study 96% of UK *N. californicus* were found to diapause and, of the other two groups of mites, only the USA ones demonstrated diapause ability. This would suggest that the UK mites originated from the USA. Given more time, work on a molecular basis for determining strain differences in this mite would have been carried out and such a study may have provided an answer for the question concerning the origin of the UK mites.

In summary, the cross-mating experiment showed that *N. californicus* from USA, Spain and UK were of the same species and reproductively compatible. Significant differences were found to occur in size of most of the morphological characteristics measured (except seta J2), in development rates, and there was a difference in band patterns when electrophoresis was

carried out. An experiment into diapause determination in *N. californicus* from these three different origins is described in chapter 4 (section 4.2). There were found to be differences in their response to short daylengths; 96% of the UK *N. californicus*, 16% of the USA and 0% of the Spanish mites underwent diapause. The present study has, therefore, found some evidence to suggest that that *N. californicus* from the three different geographical origins are distinct strains as they display different, distinguishable characteristics.

FINAL DISCUSSION

There is no evidence to show that *Neoseiulus californicus* is a native phytoseiid mite; it was not collected during the Chant (1959) survey of phytoseiids in the UK. However, it was released at a number of sites during the early 1990's. Since no records exist of *N. californicus* in the UK, that predate its first release, it can be inferred that those found in the late 1990's derive from these releases. The aims of this project were:

1. to determine whether *N. californicus* has established in the UK
2. to assess its potential as a biocontrol agent for *Panonychus ulmi*

The taxonomy of *N. californicus* was confused, so the first part of the project concentrated on unravelling the descriptions and synonymies that had previously been made. Before establishing its occurrence in the UK, it was necessary to clarify which species it was synonymous with and elucidate a clear description of the mite to enable correct identifications to be made. It was concluded that *N. californicus* is synonymous with *Amblyseius californicus* (Schuster & Pritchard, 1963), *A. chilensis* (Athias-Henriot, 1977) and *T. mungeri* (McGregor, 1954). Drawings were made and measurements of certain morphological characteristics were taken for all stages of *N. californicus*. There are no previously published drawings or measurements for immature stages of *N. californicus*. In addition, some novel observations were made. The immature stages all have eye-shaped pores in the ventrianal shield; this has not been previously noted and allows easier identification of immature stages of this mite. The pores in the ventrianal shield are openings in the shield of the mite, and not merely folds in the cuticle. This has interesting implications; what might be the function of such pores and why do some phytoseiid mites have them and others do not?

In field collections of plant material, *N. californicus* was found in strawberry, blackcurrant, hop and apple in the main fruit growing regions of the UK. It was found to possess the ability to diapause, is a chill tolerant species and was found to survive winter field conditions. As it has been found to occur on a range of host plants, and is able to survive the winter it can be concluded that it has become established in the UK. This may prompt a request for the re-

licensing of this species for unprotected release as a biocontrol agent. Although it seems certain that this species is now established, it would be necessary to conduct studies into the impact of large-scale releases of this mite on native fauna before endorsing this. The most obvious potential disadvantage is that it might attack other useful biocontrol agents, such as other phytoseiids. However, there are other possible disadvantages that may be less apparent. For example, the consumption of phytophagous mites could result in the decrease in a food resource for other phytoseiids, which may be more effective as long-term biocontrol agents. DETR may also be concerned with the introduction of a predator that has the potential to consume non-target organisms as, even though there may be no financial implications, there could be an impact on biodiversity.

The question of whether an organism can survive winter field conditions is a complicated one. This is a broad subject which is being investigated by Bale's group (Bale, 1987; Bale, 1996; Bale & Walters, 1997). It is evident that *N. californicus* freezes at temperatures that are not likely to limit its survival in our climate. However, the effect of long periods of exposure to low temperatures higher than the freezing point of an organism are harder to quantify. It is a challenging task to attempt to define protocols and standard procedures to test the response of an organism to low temperatures and to relate that information to field circumstances. The super cooling point (SCP) measured for *N. californicus* was between -19.9°C and -23.4°C. At brief (1h) exposure to -10°C, 100% survival was obtained. 20-30% *N. californicus* reared in long daylengths survived six days exposure at -7°C, and all were dead after eight days. When reared in short daylengths, survival increased to 50-70% after 6 days exposure. In the last ten years the minimum air temperature recorded at HRI-East Malling has fallen below -5°C on only 30 days in total, and the longest period of time it remained at -5°C for was 5 consecutive days in 1991. The minimum air temperature has fallen below -10°C on only 45 occasions in the last 76 years. The greatest continual period of time for which minimum temperatures of <-10°C were recorded was three days in 1940 and 1985. As *N. californicus* can survive exposure for one hour at -10°C and shows approximately 50% survival rate for 6 days exposure at -7°C, it can be inferred that there is no evidence to support the view that *N. californicus* will not survive our winter.

The ability to diapause is more likely to increase the chance of winter survival. For instance, *T. pyri* (a native phytoseiid mite) undergoes diapause and successfully survives winter field conditions (Fitzgerald & Solomon, 1991) whereas *Phytoseiulus persimilis* (a non-native phytoseiid which is released in unprotected crops in the UK for control of *Tetranychus urticae*) cannot undergo diapause (Morewood, 1993) and does not survive the winter. The idea that diapause is necessary to successfully overwinter is supported by the fact that conditions for inducing diapause differ for mites from different geographical areas (Croft, 1971; Veerman, 1992; Bondarenko & Hai-Yuan, 1958). The fact that diapause in mites from regions of different latitude and altitude is triggered by different daylength and/or temperature thresholds shows that there is an evolutionary pressure to respond to the conditions peculiar to that region, and thus enter a state that best enables winter survival. The findings of the present study, which show that *N. californicus* of different geographical origins display different diapause propensities, strongly suggests that diapause plays a role in winter survival for this species. The average daily minimum temperature, for 1931-1960, in December and January was 1.66°C and 0.48°C respectively in HRI, East Malling, UK. In Bakersfield, S. California the December and January average, for the same period of years, was 3.6°C and 3.0°C respectively. In Valencia, Spain the average was 6.7°C and 5.6°C respectively. In the present study, the percentage diapause of mites from these regions was 0%, 16% and 96% for Valencia (Spain), S. California and UK respectively. Thus, *N. californicus* from the country with the lowest minimum temperatures (UK) showed the greatest diapause propensity, whereas those from the country with the highest minimum temperatures showed the least diapause propensity (Spain). It should be noted that these minimum temperatures are averaged over long periods of time and are recorded at one weather station in each country only. Temperatures will vary greatly across countries and even within habitats. However, it is interesting to note that there is an apparent inverse relationship between the percentage diapause recorded in the current study and the minimum temperatures of the countries involved. Starvation may influence winter mortality. Diapause may allow mites to survive when food availability is low; less energy is needed if egg laying has ceased. Morewood & Gilkeson (1991) found that the body contents of diapausing *Amblyseius cucumeris* were granular in appearance, which could be due to the accumulation of lipids as energy reserves. However, there is not sufficient information available about the overwintering capability of

phytoseiids as a group to be able to make such a generalisation and it is possible that for some species diapause will increase the chance but not be an essential component of survival in the UK climate. The ability of *N. californicus* to diapause will make the case for re-licensing harder. If no diapause ability had been found then there would be little reason to refuse the licence as its chance of surviving the winter is negligible, as is the case for *P. persimilis*. There is evidence from the LTime experiments that diapausing *N. californicus* are better able to withstand low temperatures than non-diapausing ones. However, it would be prudent to investigate thoroughly the overwintering potential of non-diapausing mites before assuming that their inability to diapause is sufficient grounds to grant a licence. Thus, this study has shown that *N. californicus* has become established in the UK and that it is able to survive UK winter field conditions.

A successful biocontrol agent has certain desirable characteristics. These include: high reproductive rate and development rate to enable swift numerical response to increasing prey density, ability to survive when prey is scarce, high power of dispersal, low tendency for cannibalism (facilitates mass-rearing), high rate of activity and ability to survive at a range of climatic conditions. *Neoseiulus californicus* has been shown to be a promising candidate that possesses many of these attributes. McMurtry & Croft (1997) have noted it as having high activity and ability to survive at a range of conditions. It demonstrates good dispersal ability (Fauvel *et al.*, 1993), is able to survive seven days starvation (Palevsky *et al.*, 1999) and shows a greater tendency for predation rather than cannibalism (Palevsky *et al.*, 1999; Schausberger & Croft, 1999). In the present study, *Neoseiulus californicus* readily consumed *P. ulmi* and *T. urticae*. It consumed greater numbers of prey than the native phytoseiid *Typhlodromus pyri* and showed faster development times than *T. pyri*. Therefore, it may show a greater numerical response to prey density than *T. pyri*.

However, at present *N. californicus* is only occurring up in low numbers and infrequently on apple trees (personal observation). It is possible that apple trees present a sub-optimal habitat for this mite in the UK climate. It has been occurring in greater numbers and with greater frequency (i.e. at more sites) on strawberry in conjunction with *T. urticae* (Easterbrook, 1998) and the tarsonemid mite *Phytonemus pallidus* (Zimmerman) (Easterbrook – personal communication),

and with *T. urticae* on hops (personal observation). In the current study, *N. californicus* developed more rapidly when feeding on *T. urticae* than when feeding on *P. ulmi*, and it may be better suited as a predator of *T. urticae*. It may transpire that *N. californicus* will play a key role in control of these mites on soft fruit (Easterbrook, Fitzgerald & Solomon, 2000) and hops (Campbell – unpublished results), rather than on top fruit. Thus, there is little optimism for the use of *N. californicus* as a biocontrol agent for *P. ulmi* on apple trees in the UK. However, there is potential for it as a control agent on other crops such as strawberry and hop.

During the course of this study it became apparent that *N. californicus* from different geographical origins displayed differences in some characteristics (diapause ability, development rate, fecundity, esterase banding patterns). This raises the question of strain differences as a factor to be considered when making decisions about suitability or licence for introduction. For example, it would be best for a biocontrol company to source their mites from a strain with faster development rates and a greater fecundity (such as the USA mites) as these would have the greatest biocontrol potential. Diapause ability is also important. A strain that shows the ability to diapause is more likely to survive the UK winter and may not need re-introducing in the spring. Equally if this mite is not granted a licence for unprotected release then a biocontrol company might want to supply a strain with little diapause ability (such as the Spanish mites) for glasshouse control near the end of the summer to minimise the risk of escapees surviving.

This study has raised a number of interesting questions, which could be pursued further. Are there two strains of *N. californicus*, due to their geographical separation, or are there more, due perhaps to pesticide exposure? Can the origin of *N. californicus* found in the UK be determined? To answer these questions molecular studies need to be carried out to characterise strains. Does the gene pool for non-diapausing strains of *N. californicus* include the potential for diapause? Can it be induced in the mites which show none at all (the Spanish strain)? Experiments to determine the effect of short daylengths over several generations on different strains of *N. californicus* should be carried out to investigate this. What would be the effect on *T. pyri* and other native mites of the release of *N. californicus* in the field? Experiments to examine interactions between *N. californicus* and native phytoseiid mites, and field experiments

investigating the effect of releases of *N. californicus* on native predatory and phytophagous mite fauna would be essential components in determining the suitability of *N. californicus* as a biocontrol agent on unprotected crops.

APPENDICES

Appendix I

Polyvinyl alcohol mountant

In a large beaker, 40ml distilled H₂O were added to 10g polyvinyl alcohol powder. The beaker was heated in a water bath and stirred constantly.

35ml lactic acid were added.

10ml glycerine were added and the solution stirred.

The solution was cooled until lukewarm, and 100g chloral hydrate added.

The solution was filtered under vacuum.

Dehydration of mites for SEM

After fixing in 3% glutaraldehyde in 0.05M phosphate buffer, pH7 the specimen was washed in buffer (0.05M phosphate buffer, pH7). The buffer was then replaced with 30% alcohol for 1h.

The 30% alcohol was removed and replaced with 50% alcohol for 2h.

The 50% alcohol was removed and replaced with 70% alcohol for 1h.

The 70% alcohol was removed and replaced with 90% alcohol for 1h.

The 90% alcohol was removed and replaced with fresh 90% alcohol for 1h.

The 90% alcohol was removed and replaced with absolute alcohol for 1h.

The absolute alcohol was removed and replaced with fresh absolute alcohol for 1h.

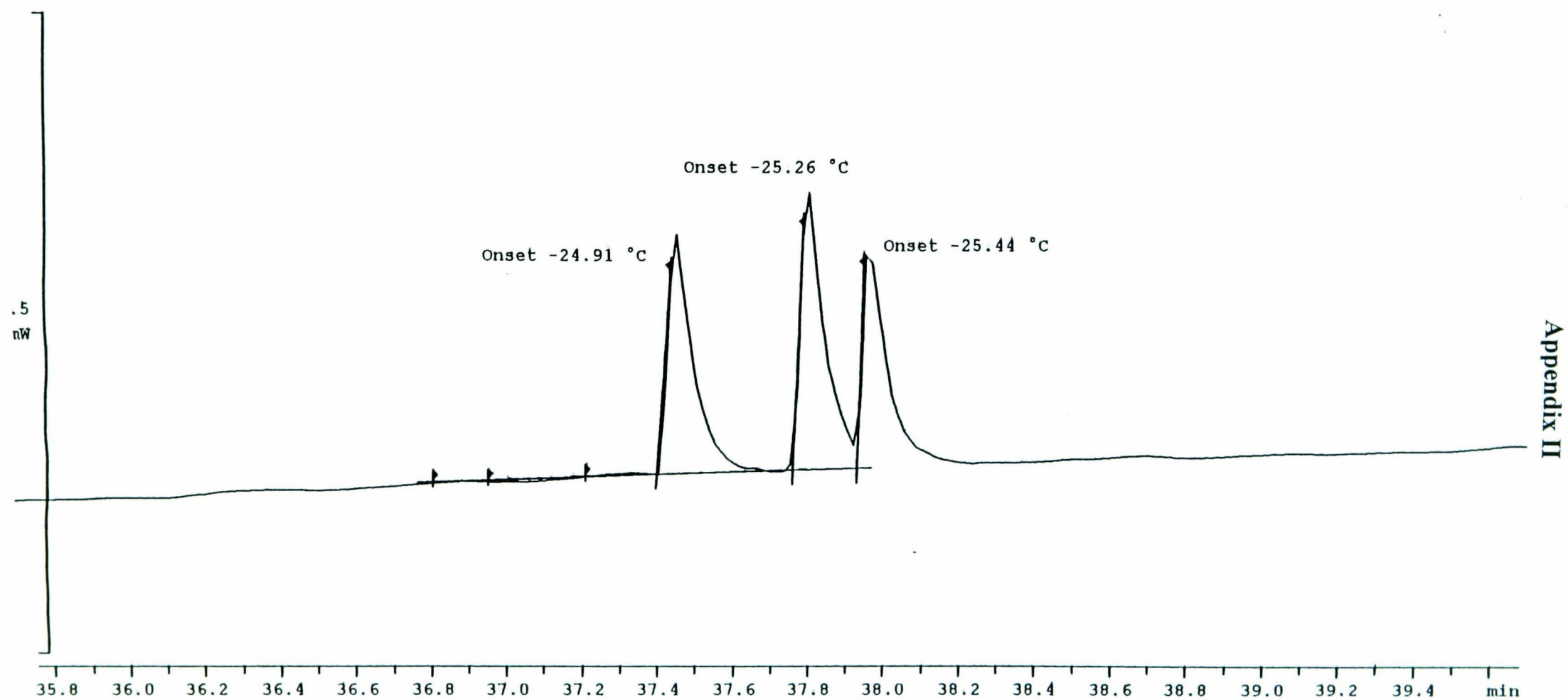
The absolute alcohol was removed and replaced with absolute alcohol: acetone (1:1) for 1h.

The alcohol:acetone was removed and replaced with acetone for 2h.

The acetone was removed and replaced with fresh acetone. This stage should be carried out for at least 12h, in the case of this experiment the mites were stored at this stage to be critical point dried and examined using the SEM a few weeks later.

Sample: HRI16, 7.3000e-03 mg

Remarks: T.pyri diapause Nos19-21 (3 individuals)



Example of trace obtained from the Mettler Toledo DSC820, evaluated by STAR^c software (version 6.1).

Appendix III

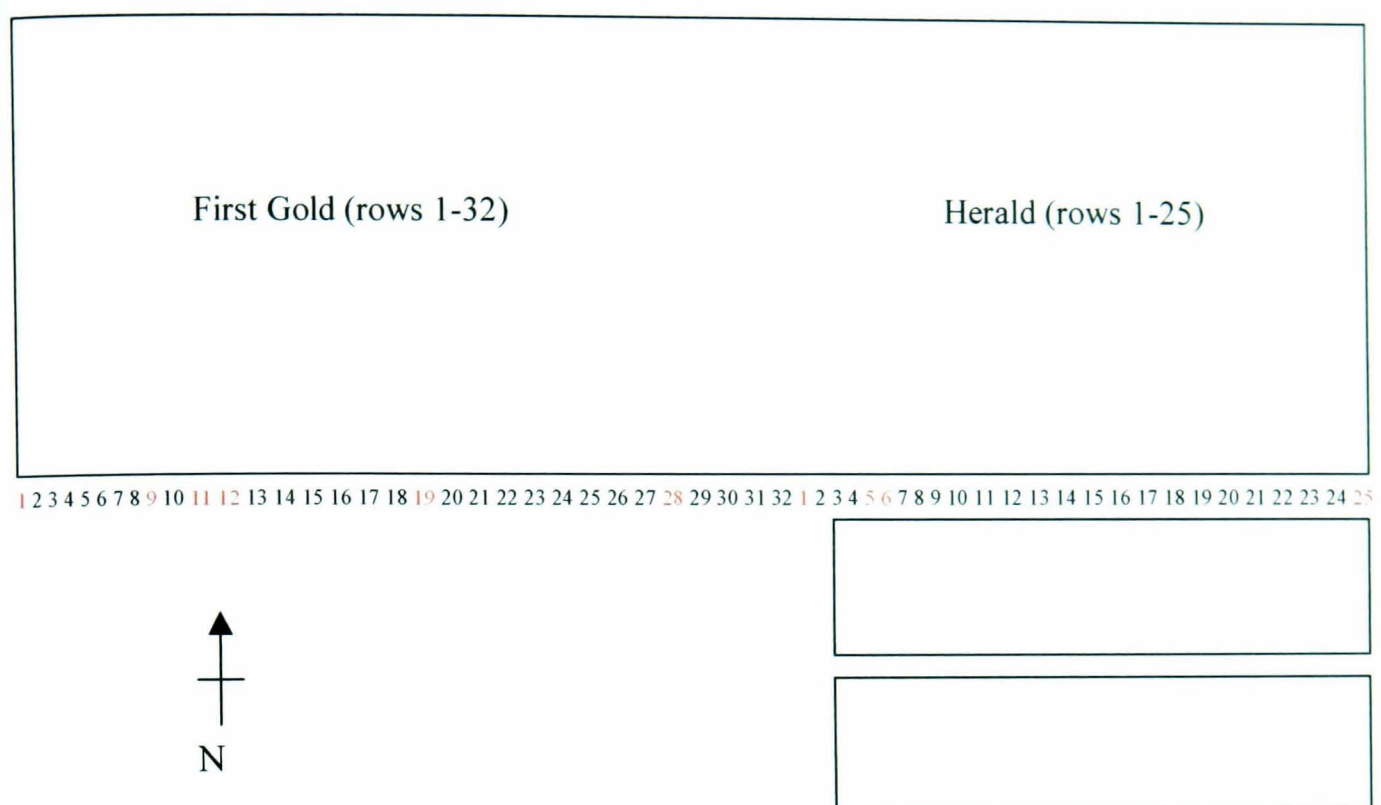


Figure II.1 Hop plot (DR149, HRI East Mallng), 1998-1999. Rows in which overwintering bands were placed are highlighted in red

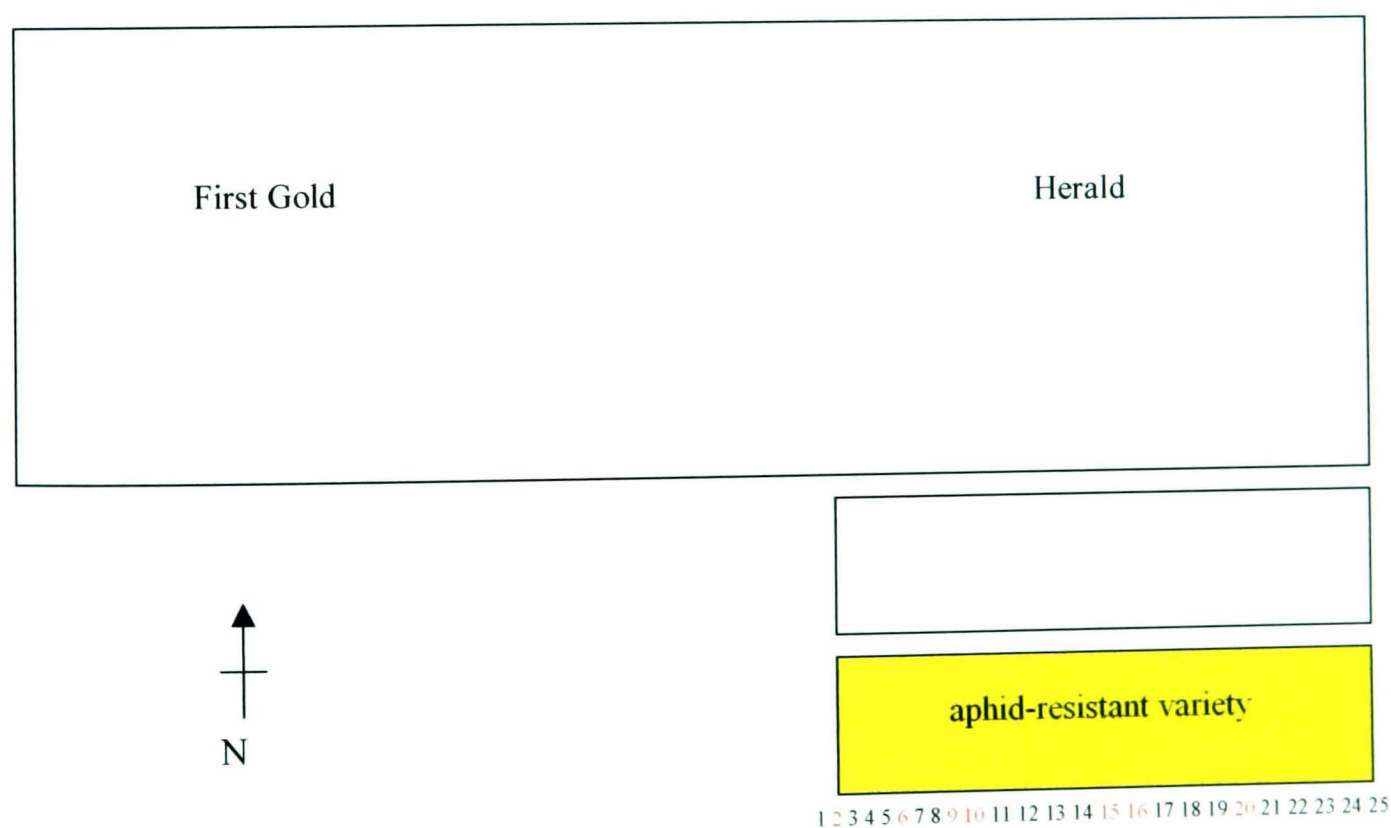


Figure II.2 Hop plot (DR149, HRI East Mallng), 1999-2000. Overwintering bands were placed in the yellow area, in the rows highlighted in red

Appendix IV

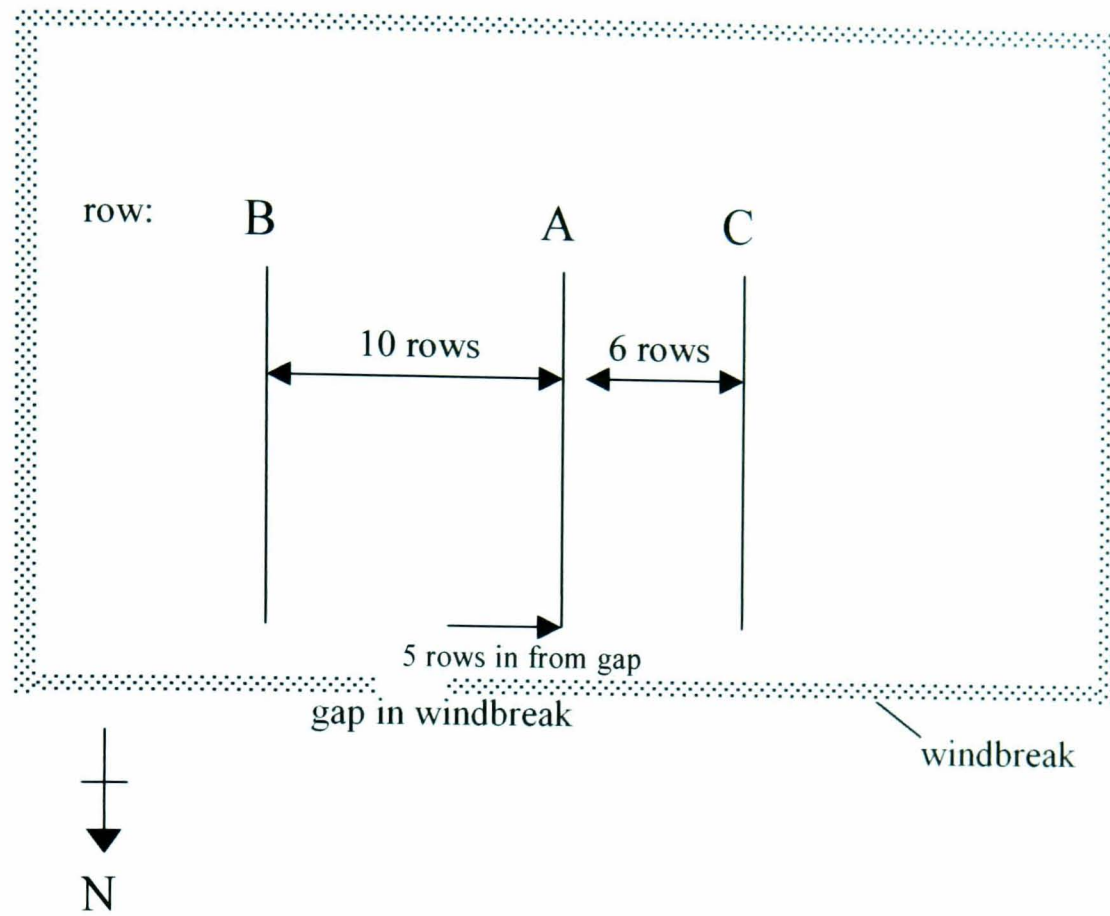


Figure III.1 Apple orchard, Stoke Fruit Farm, East Anglia, 1998-1999. Bands were placed in rows A, B and C.

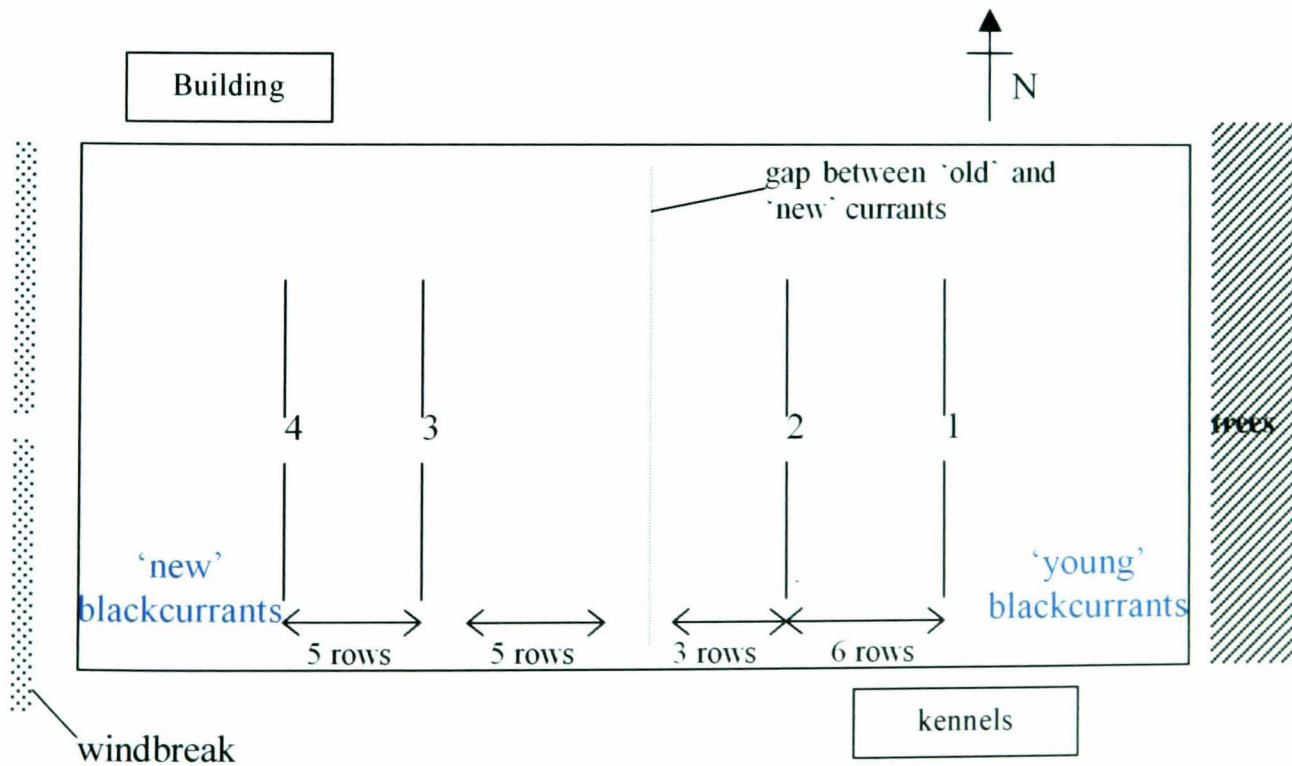


Figure III.2 Blackcurrant plot, Upper Horton Farm, Chartham Hatch, Kent, 1998-1999. Bands were placed in rows 1,2, 3 and 4.

Appendix V

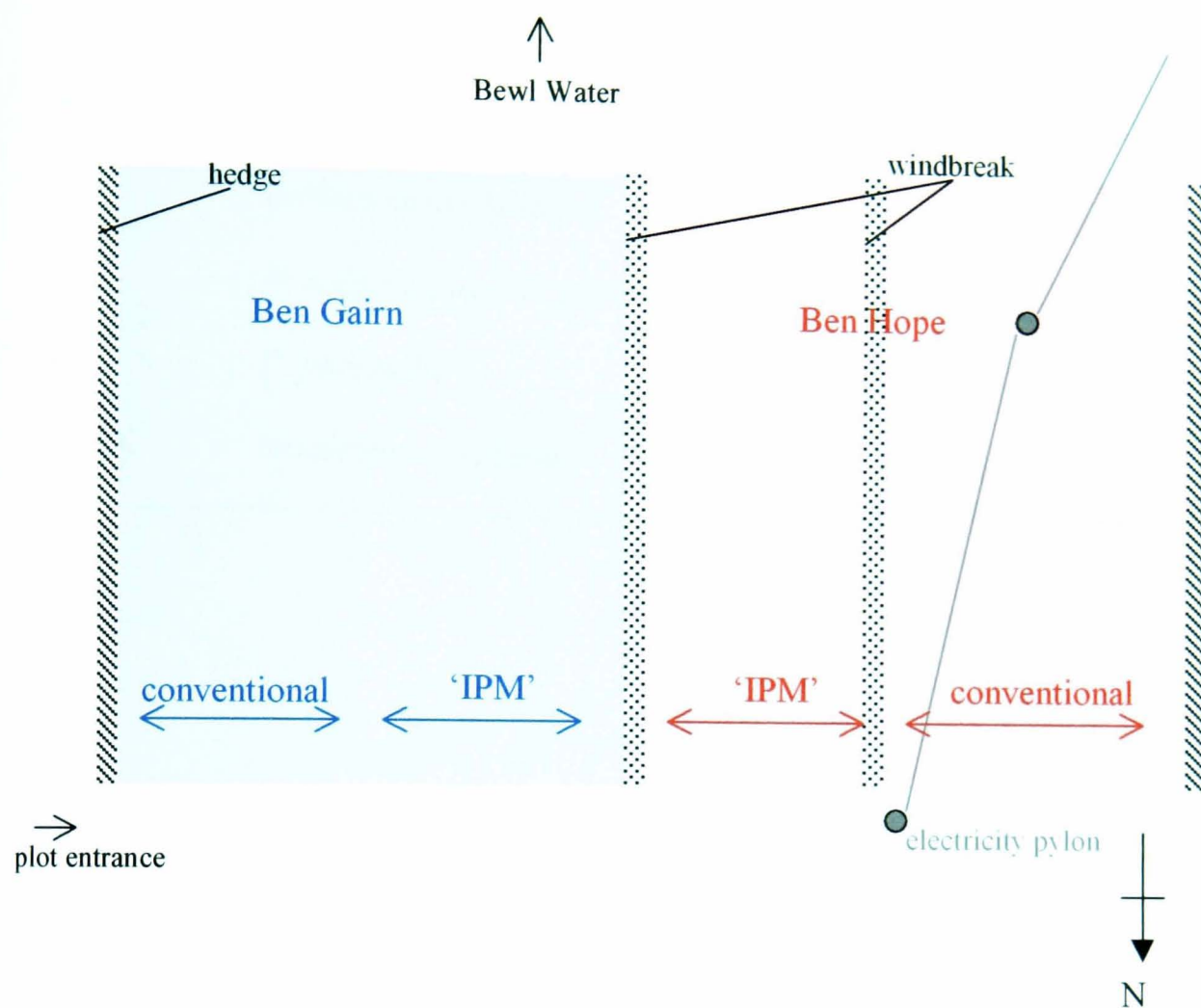


Figure IV.1 Blackcurrant plot, Rosemary Lane Ticehurst, Kent, 1999-2000. Rows ran north to south. Bands were placed in the conventional rows.

Appendix VI

Plot treatments in hop garden, HRI-EM, 1998-99.

plot number	treatment	for control of:
A1	imidacloprid (Admire)	damson hop-aphid (<i>Phorodon humili</i> (Schrank))
A3	<i>N. californicus</i> + <i>P. persimilis</i>	two-spotted spider mite (<i>T. urticae</i>)
C2	<i>P. persimilis</i>	two-spotted spider mite (<i>T. urticae</i>)
1930C	tebufenpyrad (Masai)	damson hop-aphid (<i>P. humili</i>) and two-spotted spider mite (<i>T. urticae</i>)

Appendix VII: Electrophoresis recipes

sample buffer (10ml)

8.0ml distilled H₂O
2.0ml 0.5M Tris-HCl pH6.8
0.5ml 0.5ml Triton X-100 (10% in H₂O)
1g sucrose

gel recipes

7.5% separating gel (0.375M Tris, pH8.8)

4.95ml distilled H₂O
2.5ml 1.5M Tris-HCl, pH8.8
2.5ml acrylamide/bis (30% stock)
5µl TEMED
50µl 10% ammonium persulphate (do not add until just before pouring gel)

4.0% stacking gel (0.125M Tris, pH6.8)

6.2ml distilled H₂O
2.5ml 0.5M Tris-HCl, pH6.8
1.3ml acrylamide/bis (30% stock)
10µl TEMED
50µl 10% ammonium persulphate (do not add until just before pouring gel)

Acrylamide/Bis (30% stock)

90g Bio-Rad preweighed acrylamide/bis 37.5:1 mixture
300ml distilled H₂O
store at 4°C in the dark for a maximum of 30 days

Tris-Hcl

0.5M Tris-HCl, pH6.8

6g Tris base
60ml distilled H₂O
adjust to pH6.8 with 1N HCl
make up to 100ml with distilled H₂O, store at 4°C

1.5M Tris-HCl, pH8.8

27.23g Tris base
80ml distilled H₂O
adjust to pH8.8 with 1N HCl
make up to 150ml with distilled H₂O, store at 4°C

5x running buffer, pH8.3

Tris base
43.2g glycine
make up to 600ml with distilled H₂O
for one electrophoresis run dilute 60ml 5x stock with 240ml distilled H₂O

Gel stain (for esterase activity)

These quantities are enough for two gels. Has to be made up fresh prior to use.

1. Make up 0.2M phosphate buffer, pH6:

A: 3.58g Na₂HPO₄·12H₂O in 50ml distilled H₂O

B: 1.56g NaH₂PO₄·2H₂O in 50ml distilled H₂O

For buffer pH6 mix 6.15ml A with 43.85ml B

2. Dissolve 100mg Fast Blue RR in 50ml 0.2M phosphate buffer

3. Make up 30mM 1-naphthyl acetate in acetone (56mg 1-naphthyl acetate in 10ml acetone) and add 2ml to the Fast Blue RR in buffer.

4. Filter. Keep in the dark whilst filtering and developing the gel.

5. Stop stain with 10% acetic acid. Gel may be left in 10% acetic acid for 24h and this will clear some of the background colour.

REFERENCES

- Amano, H. 1994. Collection record of predacious phytoseiid mites in Chiba prefecture (Acari Phytoseiidae). *Technical Bulletin of Faculty of Horticulture Chiba University*. **48**, 237-240.
- Arretz, V.P., Lamborot, C.L. & Araya, C.J. 1976. Strawberry pests II: a study of the problems and control of *Tetranychus urticae* [Koch]. *Investigacion Agricola (Santiago)*. **2**, 75-79.
- Athias-Henriot, C. 1959. Acariens planticoles d'Algérie. I. 5^e contribution au genre *Amblyseius* Berlese (Phytoseiidae). II. Première liste d'Actinochitinosi (Cheyletidae, Caligonellidae, Hemisarcoptidae). *Bulletin de l'Académie Royal Belgique. Classe des Sciences*. **45**, 130-153.
- Athias-Henriot, C. 1977. Nouvelles notes sur les *Amblyseiini*. III. Sur le genre *Cydnodromus*: redéfinition, composition (Parasitiformes, Phytoseiidae). *Entomophaga*. **22**, 61-73.
- Athias-Henriot, C. 1978. Définition de *Dictyonotus* nov. gen., avec description de deux espèces nouvelles de France méridionale [Gamasides, Phytoseiidae]. *Entomophaga*. **23**, 189-194.
- Auger, P., Tixier, M-S., Kreiter, S. & Fauvel, G. 1999. Factors affecting ambulatory dispersal in the predaceous mite *Neoseiulus californicus* (Acari: Phytoseiidae). *Experimental and Applied Acarology*. **23**, 235-250.
- Baillod, M., Bals, I., Linder, C., Antonin, P., Terrettaz, R. & Mittaz, C. 1995. Study of microfauna, research on predators and integrated control of the yellow spider mite *Tetranychus urticae* Koch in strawberry production. *Revue Suisse de Viticulture, d'Arboriculture et d'Horticulture*. **27**, 85-90.
- Bakker, F.M., Klein, M.E., Mesa, N.C. & Braun, A.R. 1993. Saturation deficit tolerance spectra of phytophagous mites and their phytoseiid predators on cassava. *Experimental and Applied Acarology*. **17**, 97-113.
- Bale, J.S. 1987. Insect cold hardiness: freezing and supercooling – an ecophysiological perspective. *Journal of Insect Physiology*. **33**, 899-908.
- Bale, J.S. 1996. Insect cold hardiness: a matter of life and death. *European Journal of Entomology*. **93**, 369-382.
- Bale, J.S. & Walters, K.F.A. 1997. Testing procedures for determining the overwintering potential of alien pests. *Final Report project PH01175*. 55pp.

- Baudry, O. & Reigne, M. 1993. Several phytoseiid species concomitance according to chemical treatments. *Annales ANPP Conference Internationale sue les Ravageurs en Agriculture*. **2**, 561-570.
- Block, W. 1994. Differential scanning calorimetry in ecophysiological research. *Acta Œcologica*. **15**, 13-22.
- Bondarenko, N.V. & Hai-Yuan, K. 1958. The peculiarities of the appearance of diapause in different geographical populations of the spider mite. *Doklady Akademii Nauk SSSR*. **119**, 295-298.
- Bourgouin, B., Reboulet, J. & Delpuech, I. 1993. Effects of the acaricide fenpyroximate on the beneficial fauna of orchards: results from the laboratory and the field. *Annales ANPP Conference Internationale sue les Ravageurs en Agriculture*. **2**, 763-770.
- Bruhn, J.C. & Beltrame, J.B. 1981. Aportes para el manejo de la arañuela roja europea, *Panonychus ulmi* (Koch) y su predator, *Amblyseius chilensis* (Dosse) en las plantaciones de manzanos de Uruguay. *Investigaciones Agronomicas, Centro de Investigaciones Agricolas 'Alberto Boeger'*. **2**, 3-8.
- Cahill, M., Byrne, F.J., Gorman, K., Denholm, I. & Devonshire, A.L. 1995. Pyrethroid and organophosphate resistance in the tobacco whitefly *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bulletin of Entomological Research*. **85**, 181-187.
- Calvitti, M. & Tsolakis, H. 1992. Phytoseiid mites collected from some herbaceous crops in Lazio (central Italy). *Redia*. **75**, 529-535.
- Castagnoli, M. & Amato, F. 1991. Laboratory studies on the interaction between the predator *Amblyseius californicus* (McGregor) (Acarina: Phytoseiidae) and its prey *Tetranychus urticae* Koch (Acarina: Tetranychidae). *Redia*. **74**, 77-75.
- Castagnoli, M. & Falchini, L. 1993. Suitability of *Polyphagotarsonemus latus* (Banks) (Acari: Tarsonemidae) as prey for *Amblyseius californicus* (McGregor) (Acari: Phytoseiidae). *Redia*. **76**, 273-279.
- Castagnoli, M. & Liguori, M. 1991. Laboratory observations on duration of copulation and egg production of three phytoseiid species fed on pollen. *Schuster, R. and P.W. Murphy. The Acari: Reproduction, development and life-history strategies; symposium, Graz, Austria, August 9-12, 1988*. 231-240.
- Castagnoli, M. & Simoni, S. 1991. Influence of temperature on population increase of *Amblyseius californicus* McGregor Acari Phytoseiidae. *Redia*. **74**, 621-640.

- Castagnoli, M. & Simoni, S. 1994. The effect of different constant humidities on egg and larvae of *Amblyseius californicus* (McGregor) (Acarina Phytoseiidae). *Redia*. **77**, 349-359.
- Castagnoli, M. & Simoni, S. 1999. Effect of long-term feeding history on functional and numerical response of *Neoseiulus californicus* (Acari: Phytoseiidae). *Experimental and Applied Acarology*. **23**, 217-234.
- Castagnoli, M., Ligouri, M. & Simoni, S. 1993. Distribution, sampling and association of soybean mites in Italy. *Redia*. **76**, 111-120.
- Castagnoli, M., Simoni, S. & Pintucci, M. 1995. Response of a laboratory strain of *Amblyseius californicus* (McGregor) (Acari Phytoseiidae) to semi-natural outdoor conditions. *Redia*. **78**, 273-282.
- Castagnoli, M., Ligouri, M., Simoni, S., Pintucci, M., Guidi, S. & Falchini, L. 1996. Observations on diapause induction in three phytoseiid (Phytoseiidae) species. *Acarology IX: Proceedings. Section 1: Behaviour and Physiological Ecology*. 9-12.
- Castagnoli, M., Ligouri, M. & Simoni, S. 1999. Effect of two different host plants on biological features of *Neoseiulus californicus* (McGregor). *International Journal of Acarology*. **25**, 145-149.
- Chant, D.A. 1956. Some mites of the subfamily Phytoseiinae (Acarina: Laelaptidae) from Southeastern England with descriptions of new species. *The Canadian Entomologist*. **88**, 26-37.
- Chant, D.A. 1958. Immature and adult stages of some British Phytoseiidae Berl., 1916 (Acarina). *Journal of the Linnean Society - Zoology*. **43**, 599-643.
- Chant, D.A. 1959. Phytoseiid mites (Acarine: Phytoseiidae). Part I: Bionomics of seven species in Southeastern England. Part II: A taxonomic review of the family Phytoseiidae, with descriptions of 38 new species. *The Canadian Entomologist*. Supp 12. 166pp.
- Chant, D.A. 1985. Systematics and Taxonomy. In: *World crop Pests: Spider Mites: their biology, natural enemies and control. Vol 1B*. Eds Helle, W & Sabelis, MW. Elsevier Science Publishers. 3-32.
- Chant, D.A. & Yoshida-Shaul, E. 1982. A world review of the *pyri* species group in the genus *Typhlodromus* Scheuten (Acari: Phytoseiidae). *Canadian Journal of Zoology*. **65**, 1770-1804.
- Çobanoğlu, S. 1993. Systematic studies on the Phytoseiidae (Acarina) species, found in the

- apple growing areas of Turkey II. *Türkiye entmoloji dergisi*. **17**, 99-116.
- Collyer, E. 1982. The phytoseiidae of New Zealand (Acarina) 1. The genera *Typhlodromus* and *Amblyseius* – keys and new species. *New Zealand Journal of Zoology*. **9**, 185-206.
- Costa-Comelles, J., Santamaria, A., Garci-Mari, F., Laborda, R. & Soto, A. 1990. Use of integrated control against *Panonychus ulmi* (Koch) in commercial apple crops. *Boletín de Sanidad Vegetal, Plagas*. **16**, 317-331.
- Costa-Comelles, J., DelRivero, J.M., Ferragut, F. & Garcia-Mari, F. 1994a. Integrated mite management in Spanish apple orchards. *Investigation Agraria, Produccion y Proteccion Vegetales*. **2**, 49-63.
- Costa-Comelles, J., Santamaria, A., Ferragut, F. & Garcia-Mari, F. 1994b. Population of mites in the vegetation cover of apple orchards. *Boletín de Sanidad Vegetal, Plagas*. **20**, 339-355.
- Croft, B.A. 1971. Comparative studies on four strains of *Typhlodromus occidentalis* (Acarina: Phytoseiidae). V. Photoperiodic induction of diapause. *Annals of the Entomological Society of America*. **64**, 962-964.
- Croft, B.A., Briozzo, J. & Carbonell, J.B. 1976. Resistance to organophosphorus insecticides in a predaceous mite, *Amblyseius chilenensis*. *Journal of Economic Entomology*. **69**, 563-565.
- Croft, B.A., Monetti, L.N. & Pratt, P.D. 1998a. Comparative life histories and predation types: are *Neoseiulus californicus* and *N. fallacis* (Acari: Phytoseiidae) similar type II selective predators of spider mites? *Environmental Entomology*. **27**, 531-538.
- Croft, B.A., Pratt, P.D., Koskela, G. & Kaufman, D. 1998b. Predation, reproduction and impact of phytoseiid mites (Acari: Phytoseiidae) on cyclamen mite (Acari: Tarsonemidae) on strawberry. *Journal of Economic Entomology*. **91**, 1307-1314.
- Croft, B.A., Luh, H-K. & Schausberger, P. 1999. Larval size relative to larval feeding, cannibalism of larvae, egg or adult female size and larval-adult setal patterns among 13 phytoseiid mite species. *Experimental and Applied Acarology*. **23**, 599-610.
- Dosse, G. 1958a. Über einige neue raubmilbenarten (Acar. Phytoseiidae). *Pflanzenschutzberichte*. **21**, 44-61.
- Dosse, G. 1958b. Die spermathecae, ein zusätzliches bestimmungsmerkmal bei raubmilben (Acar., Phytoseiidae). *Pflanzenschutzberichte*. **1-2**, 1-11.

- de Moraes, G.J. & McMurtry, J.A. 1985. Comparison of *Tetranychus evansi* and *T. urticae* (Acari: Tetranychidae) as prey for eight species of phytoseiid mites. *Entomophaga*. **30**, 393-397.
- de Moraes, G.J. & Mesa, N.C. 1988. Mites of the family Phytoseiidae (Acari) in Columbia, with descriptions of three new species. *International Journal of Acarology*. **14**, 71-88.
- Duso, C. & Camporese, P. 1991. Developmental times and oviposition rates of predatory mites *Typhlodromus pyri* and *Amblyseius andersoni* (Acari: Phytoseiidae) reared on different foods. *Experimental and Applied Acarology*. **13**, 117-128.
- Easterbrook, M.A. 1998. The beneficial fauna of strawberry fields in south-east England. *Journal of Horticultural Science and Biotechnology*. **73** (1), 137-144.
- Easterbrook, M.A., Fitzgerald, J.D. & Solomon, M.G. 2000. Biological control of strawberry tarsonemid mite *Phytonemus pallidus* and two-spotted spider mite *Tetranychus urticae* on strawberry in the UK using species of *Neoseiulus* (*Amblyseius*) (Acari: Phytoseiidae). *In press*.
- Ehara, S. 1966. A tentative catalogue of predatory mites of Phytoseiidae known from Asia, with descriptions of five new species from Japan. *Mushi*. **39**, 9-30.
- Ehler, L.E. 1992. Guild analysis in biological control. *Environmental Entomology*. **21**, 26-40.
- El-Banhawy, E.M. 1976. A new predacious mite of the genus *Typhlodromus* Scheuten from Brazil. *Review of Brasil Biology*. **36**, 531-534.
- El-Banhawy, E.M. 1979. Records on phytoseiid (Acari) mites of Peru. *International Journal of Acarology*. **5**, 111-116.
- Espinha, I.G., Costa-Comelles, J., Torres, L.M. & Ferragut, F. 1995. Contribution to the knowledge of Phytoseiid mites associated with apple in northeast Portugal. *Boletim de Sanidad Vegetal, Plagas*. **21**, 223-230.
- Evans, G.O. 1987. The status of three species of Phytoseiidae Acari described by Carl Willmann. *Journal of Natural History*. **21**, 1461-1468.
- Fauvel, G. & Bourgouin, B. 1993. Present state of tolerance to insecticides and acaricides in the populations of *Neoseiulus californicus* McGregor (Acari: Phytoseiidae) from apple orchards of southern France. *Annales ANPP Conference Internationale sue les Ravageurs en Agriculture*. **2**, 789-798.
- Fauvel, G., Bourgouin, B., Perron, G. & Rouzet, J. 1993. Extent of the colonization of apple

- and peach orchards in southern France by *Neoseiulus californicus* and the consequence for the biological control of *Panonychus ulmi* (Acari: Phytoseiidae, Tetranychidae). *Ann. ANPP Conference Internationale sue les Ravageurs en Agriculture*. **2**, 587-598.
- Ferreira, M.A. & Carmona, M.M. 1994. Acarofauna of Phaseolus in Portugal. *Boletin de Sanidad Vegetal, Plagas*. **20**, 111-118.
- Fitzgerald, J.D. 1999. Resistance to organophosphorous insecticides in the predatory mite *Typhlodromus pyri* Scheuten (Acarina: Phytoseiidae), an important biocontrol agent in apple orchards. *PhD thesis, University of Southampton*.
- Fitzgerald, J.D. & Solomon, M.G. 1991. Diapause induction and duration in the phytoseiid mite *Typhlodromus pyri*. *Experimental and Applied Acarology*. **12**, 135-145.
- Flechtmann, C.H.W. & McMurtry, J.A. 1992. Studies of cheliceral and deutosternal morphology of some Phytoseiidae Acari Mesostigmata by scanning electron microscopy. *International Journal of Acarology*. **18**, 163-169.
- Friese, D.D. & Gilstrap, F.E. 1982. Influence of prey availability on reproduction and prey consumption of *Phytoseiulus persimilis*, *Amblyseius californicus*, and *Metaseiulus occidentalis* (Acarina: Phytoseiidae). *International Journal of Acarology*. **8**, 85-89.
- Fournier, D., Pralavorio, M. & Pourriere, O. 1985. Study of the phytoseiid *Cydnodromus chilensis* to use against *Tetranychus urticae* in glasshouses. *Entomophaga*. **30**, 113-120.
- García-Marí, F. & González-Zamora, J.E. 1999. Biological control of *Tetranychus urticae* (Acari: Tetranychidae) with naturally occurring predators in strawberry plantings in Valencia, Spain. *Experimental and Applied Acarology*. **23**, 487-495.
- García-Marí, F., Santaballa, E., Ferragut, F., Marzal, C., Colomer, P. & Costa, J. 1983. The red mite *Panonychus citri* (McGregor): its bearing on the phytosanitary problems in our citrus groves. *Boletin del Servicio de Defensa contra Plagas e Inspeccion Fitopatologica*. **9**, 191-218.
- García-Marí, F., Ferragut, F., Marzal, C., Laborda, R., Costa-Comelles, J., Coscolla, R. & Sanchez, J. 1987. Phytoseiid and Tetranychid mite populations in Valencia, Spain, vineyards. *Investigation Agraria Produccion y Proteccion Vegetales*. **2**, 89-96.
- García-Marí, F., Gonzalez-Zamora, J.E., Orenga-Royo, S., Saques-Fernandez, J., Laborda-Cenjor, R., Soto-Sanchez, A., Ribes-Koninckx, A., Mari, F.G., Zamora, J.E.G., Royo, S.O., Fernandez, J.S., Cenjor, R.L., Sanchez, A.S. & Koninckx, A.R. 1991. Spatial

- distribution between phytophagous mite species (Tetranychidae) and predators (Phytoseiidae) on strawberry leaves. *Boletín de Sanidad Vegetal, Plagas*. **17**, 401-415.
- Garman, P. 1948. Mite species from apple trees in Connecticut. *Bulletin of the Connecticut Agricultural Experimental Station*. **520**.
- Giganti, H.E. 1993. Field evaluation of Acrinathrin 15 CE, a new acaricide insecticide for pomme fruit trees. *Revue Facultad de Agronomía*. **14**, 203-213.
- Gilstrap, F.E. & Friesse, D.D. 1985. The predatory potential of *Phytoseiulus persimilis*, *Amblyseius californicus* and *Metaseiulus occidentalis* Acarina Phytoseiidae. *International Journal of Acarology*. **11**, 163-168.
- Gilstrap, F.E., Friesse, D.D., Summy, K.R. & Armstrong, A.A. 1977. Persistence of *Amblyseius californicus*, *Phytoseiulus persimilis* and *Typhlodromus occidentalis* released in field populations of banks grass mite on corn. *Southwestern Entomologist*. **2**, 159-163.
- Gonzalez, R.H. 1971. Biología, ecología y control natural de la araña roja europea, *Panonychus ulmi* (Koch), en manzanos y perales de Chile central. Proceedings of the First Latin-American Congress of Entomology: *Revista Peruana de Entomología*. **14**, 56-65.
- Gonzalez, R.H. & Schuster, R.O. 1962. Especies de la familia Phytoseiidae en Chile I. Acarina: Mesostigmata). *University of Chile, Facultad de Agronomía, Estación Experimental Agronomía, Bolletín Técnico*. **16**, 3-25.
- Grafton-Cardwell, E.E., Ouyang, Y. & Striggo, R.A. 1997. Predaceous mites (Acari: Phytoseiidae) for control of spider mites (Acari: Tetranychidae) in nursery citrus. *Environmental Entomology*. **26**, 121-130.
- Greathouse, E.C. 1997. The role of *Zetzellia mali* (Ewing) (Acarina: Stigmaeidae) and tydeid mites in biocontrol in apple orchards. *PhD thesis, Imperial College of Science, Technology and Medicine*.
- Greco, N.M., Liljesthröm, G.G. & Sánchez, N.E. 1999. Spatial distribution and coincidence of *Neoseiulus californicus* and *Tetranychus urticae* (Acari: Phytoseiidae, Tetranychidae) on strawberry. *Experimental and Applied Acarology*. **23**, 567-580.
- Guilleminot, R. A. & Apablaza, J.U. 1986. Insects and arachnids associated with raspberry (*Rubus idaeus*) in the metropolitan region, Chile. *Ciencia e Investigación Agraria*. **13**, 251-256.
- Hale, W.G. & Margham, J.P. 1988. Collins dictionary of biology. *HarperCollins Publishers*.

- Hardman, J.M. & Rogers, M.L. 1991. Effects of temperature and prey density on survival, development, and feeding rates of immature *Typhlodromus pyri* (Acari: Phytoseiidae). *Environmental Entomology*. **20**, 1089-1096.
- Hayes, A.J. & McArdle, B.H. 1987. A laboratory study on the predatory mite *Typhlodromus pyri* (Acarina: Phytoseiidae): I. The effect of temperature and food consumption on the rate of development of the eggs and immature stages. *Researches on Population Ecology*. **29**, 73-83.
- Heller, J.J., Alibert, F., Soubrier, G. & Roa, L. 1992. Acrinathrin, a novel miticide for crop protection. *Mededelingen Faculteit Landbouwwetenschappen Rijksuniversiteit (Gent)*. **57**, 931-939.
- Hendrickson, R.M. 1980. Continuous production of predacious mites in the greenhouse. *Journal of the New York Entomological Society*. **88**, 252-256.
- Hoddle, M.S., Aponte, O., Kerguelen, V. & Heraty, J. 1999a. Biological control of *Oligonychus perseae* (Acari: Tetranychidae) on avocado: I. Evaluating release timings, recovery and efficacy of six commercially available phytoseiids. *International Journal of Acarology*. **25**, 211-219.
- Hoddle, M.S., Robinson, L. & Virzi, J. 1999b. Biological control of *Oligonychus perseae* (Acari: Tetranychidae) on avocado: III. Evaluating the efficacy of varying release rates and release frequency of *Neoseiulus californicus* (Acari: Phytoseiidae). *International Journal of Acarology*. **26**, 203-214.
- Holling, C.S. 1961. Principles of insect predation. *Annual Review of Entomology*. **6**, 163-182.
- Hoy, M.A. 1994. Insect molecular genetics. *Academic Press Inc. ISBN 0-12-357490*.
- Hoy, M.A. & Flaherty, D.L. 1975. Photoperiodic induction of diapause in a predaceous mite, *Metaseiulus occidentalis*. *Annals of the Entomological Society of America*. **63**, 960-963.
- Hsaio, S.N. 1988. Studies on the biological control of tea pests in Taiwan. In: *Recent development in tea production*. Eds. Chiu, T. F. & Wang, C. H. 149-160.
- Iraola, V.M., Biurrun, R., Moraza, M.L. & Esparaza, M.J. 1994. Predators of the family Phytoseiidae on red spider mite *Panonychus ulmi* (Koch) in orchards of Navarra. *Boletin de Sanidad Vegetal, Plagas*. **20**, 687-694.
- James, D.G. 1988. Reproductive diapause in *Typhlodromus occidentalis* Nesbitt (Acarina: Phytoseiidae) from southern New South Wales. *Journal of the Australian Entomological Society*. **27**, 55-59.

- Kerguelen, V. & Hoddle, M.S. 1999. Biological control of *Oligonychus perseae* (Acari: Tetranychidae) on avocado: II. Evaluating the efficacy of *Galendromus helveolus* and *Neoseiulus californicus* (Acari: Phytoseiidae). *International Journal of Entomology*. **25**, 221-229.
- Kielkiewicz, M. & Witul, A. 1995. Effectiveness of integrated control of spider mites on gerbera. *Proceedings of the XXXV Scientific Meeting of the Institute of Plant Protection*. **35**, 133-137.
- Knight, J.D. & Bale, J.S. 1986. Cold hardiness and overwintering of the grain aphid *Sitobion avenae*. *Ecological Entomology*. **11**, 189-197.
- Kreiter, S. & de la Bourdonnaye. 1993. Les typhlodromes, acariens prédateurs: clé simplifiée d'identification des principales espèces des cultures de plein champ en France. *Les cahiers de Phytoma – La défense des végétaux*. **4**, Supp 446. I-XI.
- Kreiter, S., Sentenac, G. & Valentin, G. 1993. The Phytoseiidae (Acari) of French vineyards. Synthesis of a 8 years survey. *Annales ANPP Conference Internationale sue les Ravageurs en Agriculture*. **2**, 597-609.
- Laing, J.E. & Osborn, J.A.L. 1974. The effect of prey density on the functional and numerical responses of three species of predatory mites. *Entomophaga*. **19**, 267-277.
- Lee R E. 1991. Principles of insect low temperature tolerance. In: *Insects at low temperature*. Eds. Lee, R. E. & Denlinger, D. L. Chapman and Hall. 17-46.
- Ligouri, M. & Castagnoli, M. 1989. Association d'acariens sur la vigne en Toscano: repartition et fluctuation des populations sur les feuilles, les écorces et les herbes au pied des ceps. In: Cavalloro R [ed.], Influence of environmental factors on the control of grape pests, diseases and weeds. *Proceedings of a Meeting of the EC Experts Group*. 181-189.
- Lindquist, E.E. & Evans, G.O. 1965. Taxonomic concepts in the Ascidae, with a modified setal nomenclature for the idiosoma of the Gamasina (Acarina: Mesostigmata). *Memoirs of the Entomological Society of Canada*. **47**, 64pp.
- Ma, W-L. & Laing, J.E. 1973. Biology, potential for increase and prey consumption of *Amblyseius chilenensis* (Dosse) (Acarina: Phytoseiidae). *Entomophaga*. **18**, 47-60.
- Mattioda, H., Heller, J.J. & Chapuis, G. 1990. Acrinathrin: a new acaricide for use in orchards. *Annales ANPP Conference Internationale sue les Ravageurs en Agriculture*. **2**, 417-424.
- McGregor, E.A. 1954. Two new mites in the genus Typhlodromus (Acarina, Phytoseiidae). *Bulletin of Southern California Academy of Sciences*. **53**, 89-92.

- McMurtry, J.A. 1977. Some predaceous mites (Phytoseiidae) on citrus in the Mediterranean region. *Entomophaga*. **22**, 19-30.
- McMurtry, J.A. 1982. The use of phytoseiids for biological control: progress and future prospects. In: Hoy M A [ed.], Recent advances in knowledge of the Phytoseiidae. *Division of Agriculture and Natural Resources, Special Publication – University of Claifornia*. 3284, 23-48.
- McMurtry, J.A & Badii, M.H. 1989. Reproductive compatibility in widely seperated populations of three species of phytoseiid mites (Acari: Phytoseiidae). *Pan Pacific Entomologist*. **65**, 397-402.
- McMurtry, J.A. & Croft, B.A. 1997. Life-styles of phytoseiid mites and their role in biological control. *Annual Review of Entomology*. **42**, 291-321.
- McMurtry, J.A., Oatman, E.R. & Fleschner, C.A. 1971. Phytoseiid mites on some tree and row crops and adjacent wild plants in Southern California. *Journal of Economic Entomology*. **64**, 405-408.
- McMurtry, J.A., Johnson, H.G. & Badii, M.H. 1984. Experiments to determine effects of predator releases on popluations of *Oligonychus punicae* (Acarina: Tetranychidae) on avocado in California. *Entomophaga*. **29**, 11-19.
- McMurtry, J.A., Johnson, H.G. & DeMoraes, G.J. 1991. Arrestment responses of some phytoseiid mites to extracts of *Oligonychus punicae*, *Tetranychus urticae* and pollen. *Israel Journal of Entomology*. **25-26**, 29-34.
- McNaughton, J. L. & Mortimer, C. T. 1975. Differential scanning calorimetry. *Perkin-Elmer Corporation*. 'Reprinted from: *IRS; Physical Chemistry Series 2*. **10**.' L-604. 44pp.
- Mesa, N.C., Braun, A.R. & Belotti, A.C. 1990. Comparison of *Mononychellus progresivus* and *Tetranychus urticae* as prey for five species of phytoseiid mites. *Experimental and Applied Acarology*. **9**, 159-168.
- Miedema, E. 1987. Survey of phytoseiid mites (Acari: Phytoseiidae) in orchards and surrounding vegetation on northwestern Europe, especially in the Netherlands. Keys descriptions and figures. *Tijdschrift over Plantziekten*. **93**, Supp 2, 1-64.
- Monetti, L.N. 1995. Seasonal dynamics of phytophagous and predatory mites (Acari: Tetranychidae, Phytoseiidae) in comercial apple orchards in Argentina, with alternative weed control practices. *Boletin de Sanidad Vegetal, Plagas*. **21**, 213-241.

- Monetti, L.N. & Croft, B.A. 1997. *Neoseiulus californicus* (McGregor) and *Neoseiulus fallacis* (Garman): Larval responses to prey and humidity, nymphal feeding drive and nymphal predation on phytoseiid eggs. *Experimental and Applied Acarology*. **21**, 225-234.
- Monetti, L.N. & Fernandez, N.A. 1995. Seasonal populations dynamics of the European red mite (*Panonychus ulmi*) and its predator *Neoseiulus californicus* in a sprayed apple orchard in Argentina (Acari: Tetranychidae, Phytoseiidae). *Acarologia*. **36**, 325-331.
- Monteiro, L.B. 1993. Biological control of *Panonychus ulmi* (Koch) (Acari: Tetranychidae) as a function of a fruit fly treatment program in the apple orchards of the region of Vacaria (Brazil). *Annales ANPP Conference Internationale sur les Ravageurs en Agriculture*. **2**, 611-620.
- Moorewood, W.D. 1992. Cold hardiness of *Phytoseiulus persimilis* Athias-Henriot and *Amblyseius cucumeris* (Oudemans) (Acarina:Phytoseiidae). *Canadian Entomologist*. **124**, 1015-1025.
- Moorewood, W.D. 1993. Diapause and cold hardiness of phytoseiid mites Acarina Phytoseiidae. *European Journal of Entomology*. **90**, 3-10.
- Moorewood, W.D. & Gilkeson, L.A. 1991. Diapause induction in the thrips predator *Amblyseius cucumeris* (Acarina: Phytoseiidae) under greenhouse conditions. *Entomophaga*. **36**, 253-263.
- Muma, M.H. 1961. Subfamilies, genera, and species of Phytoseiidae (Acarine: Mesostigmata). *Bulletin of the Florida State Museum, Biological Sciences*. **5**, 267-302.
- Müther, J., Raffo Benegas, M.D. & Vogt, H. 1996. On the importance of the predatory mite *Neosieulus californicus* (McGregor) (Acari, Phytoseiidae) in apple orchards of the province Rio Negro/Argentina. *Mitteilungen aus der Biologischen Bundesanstalt für Land und Forstwirtschaft*. **321**, 610-611.
- Murray, R.A. & Solomon, M.G. 1985. A micro-technique for preparing homogenates of biological samples. *Analytical Biochemistry*. **151**, 400-402.
- Murray, R.A., Solomon, M.G. & Fitzgerald, J.D. 1989. The use of electrophoresis for determining patterns of predation in arthropods. In: *Electrophoretic studies on Agricultural Pests*. Eds. Loxdale, H. & den Hollander, J. Clarendon Press, Systematics Association Special Volume. **39**, 467-483.
- Mwambula, J.D. 1998. Biological control of *Tetranychus urticae* using phytoseiid predatory

- mites, *Amblyseius californicus* and *Phytoseiulus persimilis*: a comparative study. *MSc report, Wye College*.
- Nicotina, M. 1996. Phytoseiid mites found in the vineyards of Lazio region, Italy. *Bolletino del Laboratorio di Entomologia Agraria Filippo Silvestri*. **52**, 117-130.
- Navajas, M., Lagnel, J., Fauvel, G. & DeMoraes, G. 1999. Sequence variation of ribosomal Internal Transcribed Spacers (ITS) in commercially important Phytoseiidae mites. *Experimental and Applied Acarology*. **23**, 851-859.
- Nesbitt, H.H.J. 1951. A taxonomic study of the Phytoseiinae (family Laelaptidae) predaceous upon Tetranychidae of economic importance. *Zoologische verhandelingen*. **12**, pp63.
- Oatman, E.R. 1971. Mite species on strawberry in Southern California. *Journal of Economic Entomology*. **64**, 1313-1314.
- Oatman, E.R., McMurtry, J.A., Gilstrap, F.E. & Voth, V. 1977a. Effect of releases of *Amblyseius californicus*, *Phytoseiulus persimilis* and *Typhlodromus occidentalis* on the twospotted spider mite on strawberry in southern California. *Journal of Economic Entomology*. **70**, 45-47.
- Oatman, E.R., McMurtry, J.A., Gilstrap, F.E. & Voth, V. 1977b. Effect of releases of *Amblyseius californicus* on the twospotted spider mite on strawberry in southern California. *Journal of Economic Entomology*. **70**, 638-640.
- Overmeer, W.P.J. 1985a. Rearing and handling. In: *World crop Pests: Spider Mites: their biology, natural enemies and control*. Vol 1B. Eds Helle, W. & Sabelis, M.W. Elsevier Science Publishers. 161-170.
- Overmeer, W.P.J. 1985b. Diapause. In: *World crop Pests: Spider Mites: their biology, natural enemies and control*. Vol 1B. Eds Helle, W. & Sabelis, M.W. Elsevier Science Publishers. 95-101.
- Overmeer W.P.J., Nelis, H.J.C.F., De Leenheer, A.P., Calis, J.N.M & Veerman, A. 1989. Effect of diet on the photoperiodic induction of diapause in three species of predatory mite, *Amblyseius potentillae*, *A. cucumeris* and *Typhlodromus pyri*. *Experimental and Applied Acarology*. **7**, 281-287.
- Palevsky, E., Reuveny, H., Okonis, O. & Gerson, U. 1999. Comparative behavioural studies of larval and adult stages of phytoseiids (Acari: Mesostigmata) *Typhlodromus athiasae* and *Neoseiulus californicus*. *Experimental and Applied Acarology*. **23**, 467-485.
- Pande, Y.D., Carnero, A. & Hernandez, M. 1989. Notes on biological observations on some

- unrecorded species of phytophagous and predatory mites in Canary Islands. *Investigacion Agraria e Produccion y Proteccion Vegetales*. **4**, 275-281.
- Peña, J.E. & Osborne, L. 1996. Biological control of *Polyphagotarsonemus latus* (Acarina: Tarsonemidae) in greenhouses and field trials using introductions of predacious mites (Acarina: Phytoseiidae). *Entomophaga*. **41**, 279-285.
- Perrot-Minnot, M-J., Lagnel, J., Migeon, A. & Navajas, M. 2000. Tracking paternal genes with DALP markers in a pseudoarrhenotokous reproductive system: biparental transmission but haplodiploid-like inheritance in the mite *Neoseiulus californicus*. *Heredity*. **84**, 702-709.
- Pickett, C.H. & Gilstrap, F.E. 1986a. Predation of *Oligonychus pratensis* Acarina Tetranychidae by *Phytoseiulus persimilis* and *Amblyseius californicus* Acarina Phytoseiidae under controlled laboratory conditions. *Entomophaga*. **31**, 205-212.
- Pickett, C.H. & Gilstrap, F.E. 1986b. Inoculative releases of phytoseiids (Acari) for the biological control of spider mites (Acari: Tetranychidae) in corn. *Environmental Entomology*. **15**, 790-794.
- Raworth, D.A., Fauvel, G. & Auger, P. 1994. Location, reproduction and movement of *Neoseiulus californicus* (Acari: Phytoseiidae) during the autumn, winter and spring in orchards in the south France. *Experimental and Applied Acarology*. **18**, 593-602.
- Rodriguez-Reina, J.M., Garcia-Mari, F. & Ferragut, F. 1992. Predatory activity of phytoseiid mites on different developmental stages of the Western flower thrips *Frankliniella occidentalis*. *Boletin de Sanidad Vegetal, Plagas*. **18**, 253-263.
- Rott, A.S. & Ponsonby, D.J. 1998. The effect of temperature, relative humidity and host plant on the behaviour of *Amblyseius californicus* as a predator of the two-spotted spider mite (*Tetranychus urticae*). *Proceedings of the 1998 Brighton Conference: Pests and Diseases*. 807-812.
- Rott, A.S. & Ponsonby, D.J. 2000. Improving the control of *Tetranychus urticae* on edible glasshouse crops using a specialist coccinellid (*Stethorus punctillum* Weise) and a generalist mite (*Amblyseius californicus* McGregor) as biocontrol agents. *Biocontrol Science and Technology*. **10**, 487-498.
- Sabelis, M.W. & Bakker, F.M. 1992. How predatory mites cope with the web of their tetranychid prey: a functional view on dorsal chaetotaxy in the Phytoseiidae. *Experimental and Applied Acarology*. **16**, 203-225.

- Saito, Y., Mori, K. & Chittenden, A.R. 1999. Body characters reflecting the body size of spider mites in flattened specimens (Acari, Tetranychidae). *Applied Entomology and Zoology*. **34**, 383-386.
- Salt, R.W. 1966. Factors influencing nucleation in supercooled insects. *Canadian Journal of Zoology*. **44**, 117-133.
- Sakata, T., Hiramatsu, N., Ishikawa, K. & Kuwahara, Y. 1997. Chemical ecology of Mesostigmatid mites. I: Identification of hydroxymethylnaphthoquinones from *Uroactinia hirschmanni* Hiramatsu (Mesostigmata: Uropodidae). *Applied Entomology and Zoology*. **32**, 57-61.
- Schausberger, P. & Croft, B.A. 1999. Predation on and discrimination between con- and heterospecific eggs among specialist and generalist phytoseiid mites (Acari: Phytoseiidae). *Environmental Entomology*. **28**, 523-528.
- Schulten, G.G.M. 1985. Pseudo-arrhenotoky. In: *World crop Pests: Spider Mites: their biology, natural enemies and control*. Vol 1B. Eds Helle, W. & Sabelis, M.W. Elsevier Science Publishers. 67-71.
- Schuster R.O & Pritchard, A.E. 1963. Phytoseiid mites of California. *Hilgardia*. **34**, 191-285.
- Sentenac, G., Kreiter, S., Weber, M., Barthes, D. & Jacquet, C. 1993. Integrated control of phytophagous mites on grapes. In: *Proceedings, International Congress Euroviti 7th, 24-25 October 1993, Montpellier, france*. 135-174.
- Simoni, S. 1992. Determination of the primary sex ratio in *Amblyseius californicus* (McGregor), *Amblyseius cucumeris* (Oud.) and *Typhlodromus exilaratus* Ragusa (Acari: Phytoseiidae). *Redia*. **75**, 467-474.
- Smith, V.A., Orr, D.B. & Hart, E.R. 1993. Economic analysis of two-spotted spider mite management on greenhouse-grown poplars. *Tree Planter's notes*. **44**, 154-156.
- Sømme, L. 1982. Supercooling and winter survival in terrestrial arthropods. *Comparative Biochemistry and Physiology*. **73A**, 519-543.
- Steiner, M.Y. & Elliott, D.P. 1983. Biological pest management for interior plantscapes. *Vegreville AB, Alberta Environmental Centre*. 30pp. AECV83-E1.
- Swirski, E., Amitai, S. & Dorzia, N. 1970. Laboratory studies on the feeding habits, post-

- embryonic survival and oviposition of the predaceous mites *Amblyseius chilenensis* Dosse and *Amblyseius hibisci* Chant (Acarine: Phytoseiidae) on various kinds of food substances. *Entomophaga*. **15**, 93-106.
- Tanigoshi, L.K. 1982. Advances in knowledge of the Phytoseiidae. In: *Recent advances in knowledge of the Phytoseiidae, Division of Agricultural Sciences, University of California, Berkeley, CA*. **3284**, 1-22.
- Tauber, M.J., Tauber, C.A. & Masaki, S. 1986. Seasonal adaptations of insects. *Oxford University press, New York*. 411pp.
- Tijerina-Chavez, A.D. 1991. Biological control of spider mites (Acari: Tetranychidae) on cotton through inoculative release of predatory mites *Metaseiulus occidentalis* and *Amblyseius californicus* (Acari: Phytoseiidae) in the San Joaquin Valley of California. *PhD Dissertation, University of California, Davis, USA*.
- Ueckermann, E.A. & Loots, G.C. 1988. The African species of the subgenera *Anthoseius* De Leon and *Amblyseius* Berlese (Acari: Phytoseiidae). *Entomology Memoirs. Department of Agriculture, Water Supply, Republic of South Africa*. **73**, 1-168.
- Van der Geest, L.P.S., Overmeer, W.P.J. & Van Zon, A.Q. 1991. Cold-hardiness in the predatory mite *Amblyseius potentillae* (Acari: Phytoseiidae). *Experimental and Applied Acarology*. **11**, 167-176.
- Van Houten. 1989. Photoperiodic control of adult diapause in the predacious mite, *Amblyseius potentillae*: repeated diapause induction and termination. *Physiological Entomology*. **14**, 341-348.
- Van Houten, Y.M. & Veenendaal, R.L. 1990. Effects of photoperiod, temperature, food and relative humidity on the induction of diapause in the predatory mite *Amblyseius potentillae*. *Experimental and Applied Acarology*. **10**, 111-128.
- Van Houten, Y..M., Overmeer, W.P.J., Van Zon, A.Q. & Veerman, A. 1988. Thermoperiodic induction of diapause in the predacious mite, *Amblyseius potentillae*. *Journal of Insect Physiology*. **34**, 285-290.
- Van Houten Y M, Van Rijn P C J, Tanigoshi L K, Van Stratum P & Bruin J. 1995. Preselection of predatory mites to improve year-round biological control of western flower thrips in greenhouse crops. *Entomologia Experimentalis et Applicata*. **74**, 225-234.
- Veerman, A. 1992. Diapause in phytoseiid mites: a review. *Experimental and Applied*

Acarology. **14**, 1-60.

- Veerman, A. 1994. Photoperiodic and thermoperiodic control of diapause in plant-inhabiting mites: a review. *Netherlands Journal of Zoology*. **44**, 139-155.
- Villaronga, P., Coisalls, J.R. & Bonet, J. 1993. Mite fauna associated to peach orchards in Lleida (Spain). *Bulletin OILB SROP*. **16**, 14-21.
- Wu, T.K., Lin, S.R. & Lo, K.C. 1985. Selectivity of acaricides to two-spotted spider mite, *Tetranychus urticae* Koch and the predatory mite, *Amblyseius californicus*. *Journal of Agricultural Research of China*. **34**, 469-476.
- Yaninek, J.S., Onzo, A. & Ojo, J.B. 1993. Continent-wide releases of neotropical phytoseiids against the exotic cassava green mite in Africa. *Experimental and Applied Acarology*. **17**, 145-160.
- Zhang, Z-Q. & Croft, B.A. 1994. A comparative life history study of immature *Amblyseius fallacis*, *Amblyseius andersoni*, *Typhlodromus occidentalis* and *Typhlodromus pyri* (Acari: Phytoseiidae) with a review of larval feeding patterns in the family. *Experimental and Applied Acarology*. **18**, 631-657.